

Can cancer at work be regulated?

In the century and a half since cancer of the scrotum among chimney sweeps was first recognized as an occupational disease, interest in (and anxiety about) occupational cancer has grown steadily. Since the Second World War, there has been continuing concern for the health of those occupationally exposed to ionizing radiation, and all governments have introduced regulations intended to limit the exposure of workpeople and thus the risk of overt disease. More recently, attention has turned to the occupational risks of the exposure of workpeople (and also the general population) to industrial chemicals. In the United States, the passage of the Occupational Health and Safety at Work Act in 1970 led to the creation of two separate organizations with responsibility in the field — the National Institute for Occupational Safety and Health (whose function is research and evaluation) and the Occupational Safety and Health Administration (which can regulate industrial practice). In Britain, legislation on the American model led in 1974 to the creation of the Health and Safety Executive and its supervisory commission. For the past three years, the Health and Safety Executive has been struggling with a regulation requiring the notification of new chemical substances (see *Nature* 16 July, p.190) but even now is unlikely to meet the deadline of 18 September decreed by the European Commission.

Nobody should be surprised. European administrations' attempts to restrict the introduction of potentially hazardous chemicals are the analogues of the regulations enshrined in the Toxic Substances Control Act of 1976, which took the best part of six years to emerge from an embattled Congress in the United States. There are several contentious issues. Chemical companies resent the cost of subjecting all new chemicals to a battery of short and long-term toxicity tests — and, with more reason, fear that too wooden an exercise of regulatory powers by the responsible agencies may deprive them of the use of a valuable intermediate even when effective precautions may be taken. A more serious difficulty, shown up by the muddled division of responsibility in the United States between the Environmental Protection Agency (responsible for the administration of the Toxic Substances Control Act) and the Occupational Safety and Health Administration is that of making sensible arrangements for the protection of the public at large, and of people occupationally exposed to larger concentrations of the same materials.

In Britain, as earlier in the United States, a further complication has arisen — the interest of the trades unions in the design of the regulations and in their administration. This development is entirely to be welcomed. Even the most diehard opponents of organized labour would agree that unions are better occupied in the protection of the health of their members than in some of the other things they do. Industry as a whole, and the community at large, can only benefit from a proper understanding by its workpeople of the occupational risks they are asked to run — and from their informed consent that risks of some magnitude, ideally small but probably not identical with zero, should be run. The time that might now be spent in Britain by the Health and Safety Executive on reconciling the interests of the trades unions and the chemical manufacturers would be well worthwhile.

Unfortunately, good sense is in danger of being overtaken by events. The Health and Safety Executive, applauded ever since its creation as a novel and autonomous entity within the British constitution, is much in need of the safety valve of which its special place deprives it — an expression of a range of prejudiced

opinion in the House of Commons. (Is the House of Lords too busy to oblige?) The second draft of its regulations on the notification of new substances is indeed needlessly onerous, too cut and dried, and the executive should take the time to produce a third, whatever they say in Brussels. The trouble, now, is that the executive has to watch out not only for the European Commission but for Mr Clive Jenkins, the secretary of the trade union called the Association of Scientific, Technical and Managerial Staffs.

Mr Jenkins's union has in the past few years taken a close and often constructive interest in problems of occupational health and safety. More than a year ago, it produced an arresting (if controversial) document about the occupational hazards of new chemicals. Ten days ago, Mr Jenkins issued a lengthy commentary on the second draft of the Health and Safety Executive's proposed notification regulations and made some cogent points. Chemical companies are indeed too defensive of their superficial financial interests, and too unwilling to let independent outsiders enquire into their affairs. The costs of complying with regulations have been systematically (that is, consistently) exaggerated. And so on. He erred only in the most rudimentary way — by supposing that there is some state of grace in which occupational risks are absolutely avoidable. His problem, and the executive's, is rather how to strike a balance between the possibly carcinogenic risk of a novel chemical and the possibility that somebody, or some group, will be out of work. Who will persuade whom of that?

Damming estuaries

The hope that there might be some other way — any other way — of generating electricity than those now used is likely to be widely paraded in the weeks ahead. The United Nations conference on alternative energy sources due to open in Nairobi next week will be an obvious forum (see also page xxx). And nobody should deride the innocent declarations of faith in windmills, water-wheels or wood (now called biomass) that there will be. There are unfortunately still too many circumstances, in still too many developing countries, in which any way of generating a modicum of mechanical power from some indigenous source is much needed. But those who assemble in Nairobi should also take to heart the message hidden in last week's report of Sir Hermann Bondi's study of the feasibility of an electricity-generating barrage across the Severn estuary, between England and Wales (see *Nature* 30 July, p.401). The conclusion of this thorough piece of work is that if British taxpayers want the kinds of benefits that a barrage across the Severn estuary would provide, they would be best advised to build nuclear power stations and not a dam.

The argument is simple but instructive. A dam across an estuary can be used to generate electricity in several obvious ways — by letting the incoming tide drive turbines in a dam, by letting water impounded at high tide run back through turbines on the ebb tide or (as in the scheme operated by Electricité de France on the estuary of the Rance since 1966) arranging that water in an estuary will drive turbines at both the ebb and flood tides. The amounts of energy that can in principle be won from such schemes are huge — the Bondi committee estimated that even the most modest of all possible dams across the Severn could yield 13 TWh of electricity, something like six per cent of present electricity demand in the United Kingdom, roughly the amount of electricity that would be



generated by the use of 7 million tons of coal a year, year in and year out. The snag is that not a single kilowatt-hour of this electricity would accrue until the whole dam across the Severn estuary had been built — a process likely to take 15 years and to cost £5,660 million without allowing for the cost of paying interest on this substantial sum of money while construction is under way. But is not any scheme that produces energy from a renewable resource, such as the slopping one way and another of water in a tidal estuary, inherently worthwhile? That is what the enthusiasts for the Severn barrage have been saying almost since the beginning of the electricity supply industry itself. Unfortunately, a careful reading of the Bondi committee's first report shows the argument to be false.

As in other industrially developed countries, the British demand for electricity is a reasonably well-defined function of the time of day and the season of the year. Inevitably, the daily pattern of demand is linked with the movement of the Earth about the Sun and not with the movement of the Moon about the Earth, with the result that maxima of demand coincide only by accident with tidal maxima. Similarly, seasonal variations bear no constant relationship to the occurrence of spring or neap tides, which are related to the relative alignment as seen from the Earth of the Sun and the Moon. None of this is surprising. The consequence is, however, that a dam across the Severn would be capable of generating electricity at times only randomly related to those at which electricity demand is greatest. Accordingly a Severn barrage (or any other tidal scheme for generating electricity) will not substantially reduce the need for building other kinds of generating plant to satisfy the maximum demand on the system. (This blunt statement is a little over-gloomy, for electricity utilities would in principle be able to plan routine maintenance of conventional plant on a lunar calendar and thus obviate the need for some capital investment.) Such benefits as there are from tidal schemes, however, consist only in the avoidance of fuel costs at conventional power stations, some of which may be shut down when barrage schemes are ready to generate electricity. Overall, the crucial question is whether, over the lifetime of a barrage, the initial capital cost will be justified by the conventional fuel (coal, oil and uranium) that would be saved.

The Bondi committee, which includes several enthusiasts for big dams, puts a brave face on this daunting prospect. On the basis of a calculation of the present value of the conventional fuel that a Severn barrage would save, it concludes that the most modest version of such a scheme would just about pay for itself. There will be much technical interest in the way in which this calculation has been carried out — and curiosity will be satisfied only when the full report of the study is published two months or so from now. Already, however, it is clear that even this calculation is over-optimistic. The committee has assumed in calculating costs and benefits that the annual rate of interest on capital is either 5 per cent or 7 per cent — much less than the cost of the money the British government is having to borrow to finance less ambitious projects than the Severn barrage. But that is only half the story. The Bondi committee, with its hand firmly on its heart, makes the honest declaration that the economic benefits of investing in nuclear power stations would be roughly twice those of investing in the Severn barrage.

None of this implies that schemes for the exploitation of renewable energy should not be examined. The Bondi study is a model of how such exercises should be carried out. But enthusiasts for the use of renewable energy resources should acknowledge that conclusions such as those that have now put paid to dreams of a barrage across the Severn are likely to afflict many other projects for generating electricity in unconventional ways. After all, the properties of a stream of electrons in an electrical conductor are independent of the source of the electrical potential that drives them. By making uneconomic judgements about the virtues of different sources, it is possible to waste vast amounts of wealth that might be used in other ways. This is not to say that the United Nations conference at Nairobi should promptly adjourn, and amalgamate with the meeting of the major electricity utilities at the next World Power Conference, for there

is much that might be done by a sober appraisal of small power sources for use in places where there is hardly any power of any kind available at present. But, in the long run, the hard economics that have ruled out the Severn barrage in Britain are likely also to apply elsewhere.

Cricket and patriotism

The British, much depressed for several years by problems in Ulster and of economic misjudgement and mismanagement, have been so curiously uplifted in the past few weeks by a royal wedding and their success in beating the Australians twice at cricket that the question must be faced whether the psychological benefits of these public spectacles can be more widely spread. For if there is the slightest chance that peoples elsewhere might be given the courage to face intractable problems — or perhaps even the excuse to forget about them for a time — by a suitable patriotic display, should not governments other than the British be ready with plans for suitable ceremonies? Precedents set by the Roman emperors, however politically prudent 2,000 years ago, would no longer be acceptable, while the chances are small that governments will value royal weddings so highly that they would embark on the constitutional upheavals necessary to introduce monarchies of their own. On the face of things, only the game of cricket is potentially adaptable to other circumstances than the British, and even there the prospects are not bright.

The difficulty with cricket is that it is not widely understood. There is, however, no reason to accept the complaint of those not subjected to an English education that the game is inherently incapable of being understood. The way in which cricket has spread through much of the British Commonwealth gives the lie to this canard; the fact that West Indian cricketers are probably now more skilled than any others is a sign that cricket could become an international vehicle for national contentment, even chauvinism. To that end, the most urgent need is for a deeper understanding of what cricket is about. It may even be necessary to simplify the rules.

The fundamental principles of cricket are by no means unique. As in baseball, one person throws a ball at another, who is equipped for his defence with a piece of wood called a bat. Superficially, cricket is more objective than baseball in that a batsman's defence is held to have been unsuccessful if the ball strikes one of three pieces of wood planted on the ground behind him, and not merely on the say-so of an umpire who has to judge whether the ball passes at the right height over an object laid in the ground. What puzzles potential devotees of cricket is that the ball-throwing act is routinely performed in each of two opposing directions and that there appears to be no limit to the number of people who can be invited to throw the ball. It is also puzzling to many that ball-throwers (called "bowlers") may legitimately bounce their projectiles on the ground in front of the hapless batsmen, thus introducing an element of disconcerting randomness into their trajectories.

Fortunately, these faults are easily remediable. One-ended cricket played on an adhesive surface (so that cricket balls could not be bounced) would plainly make it possible for others than the Old Commonwealth to understand and thus to enjoy a game to which they are at present denied access. The time taken by cricket matches could be reduced if batsmen were given less substantial pieces of wood. For obvious economic reasons, it would be necessary to reduce the duration of international matches from five days to five hours, which to a first approximation would require that the presenting surface of the standard bat should be reduced by approximately five-sixths. The disadvantage that it would then be difficult for batsmen to accumulate large individual scores — one of the prerequisites of national cricket heroes — could be offset by multiplying by six the present rewards for batsmanship. No doubt there would be many in places where cricket is understood who would resist these innovations. But should tradition be allowed to stand in the way of a wider distribution of the benefits that cricket might bring?

Stanford sells gene-splicing licences

Boyer, Cohen patent may yet be challenged

Washington

In a move expected to bring in annual royalty payments of over a million dollars within the next four or five years, Stanford University announced this week the terms under which it is prepared to let commercial companies use the gene-splicing techniques developed in the early 1970s by Dr Stanley Cohen of the university's medical school and Dr Herbert Boyer of the University of California in San Francisco.

The techniques have since become the basic tools for many widely used genetic engineering processes. University researchers who wish to use them may continue to do so free of charge, but companies marketing products based on their use will be required to pay a royalty fee varying from one per cent of net product sales below \$5 million to 0.5 per cent on annual sales of over \$10 million.

Before reaching the commercial stage, any company wishing to use the techniques in its research can purchase a non-exclusive licence from Stanford for an initial fee of \$10,000, and an annual sum of the same amount. An incentive offered to those who act quickly is that any company which signs up before the end of 1982 can credit five times the licence fee against future royalties, up to a maximum of 50 per cent of the royalties earned in any one year.

Stanford will deduct 15 per cent from fees and royalties earned to servicing the licensing arrangements. After that the remaining sum will be divided equally between Stanford and the University of California. Stanford's share will be further divided, with a third going to the university's medical school and a third to its departments of medicine and genetics. Dr Cohen, who holds joint appointments in the two departments and who would under the university's normal rules be entitled to the remaining third, has assigned his share back to the university, to be divided between biomedical research and support for postdoctoral fellowships. Dr Boyer has also waived any rights to personal royalties.

Despite being one of the leading research universities in the United States, with an annual research budget of over \$100 million, Stanford has so far lacked any major money-spinning patents comparable, for example, with the widely-used dental fluoride developed at the University of Indiana. University officials are hoping that the income from the patent on the Cohen/Boyer techniques, filed in

1974 and granted last December, will change this, although president Donald Kennedy says he does not anticipate that royalty income from this source "will significantly alter our present financial projections".

The patent granted by the Patent Office is broad, covering the series of steps used by Drs Cohen and Boyer to replicate and express exogenous genes in micro-organisms. It describes the techniques used to cleave a plasmid or virus DNA, to insert a new gene to provide a biologically functional replicon, to place the latter in a microorganism cell, and finally to isolate the transformants in order to produce cells in which the DNA molecules in the modified plasmid can be replicated and expressed.

The breadth of the patent — and the ubiquity of the techniques' current usage — means the university is expecting a large number of licensing requests. In a statement issued on Monday it estimated that more than 200 companies in the United States may already be involved, and that half may sign up for the licences in the near future.

At the same time, this breadth could lay the patent open to challenge from other scientists who claim to have made significant contributions to the results which were patented by the universities solely in the names of Cohen and Boyer. Murmurs of possible legal challenges have surfaced in the past, although the relatively low licence fee — as well as the fact that the royalties will go to the universities and not to the individual scientists — may well dampen any conflict.

Stanford is requiring that any company obtaining a licence must agree to use the techniques in compliance with the guidelines for physical and biological containment of experiments drawn up by the National Institutes of Health.

The university has also welcomed a recent ruling by the International Trade Commission forbidding the unlicensed import of goods made abroad with techniques patented in the United States. Previously it had been feared that companies might try to avoid the licensing fee and royalty payments by setting up production facilities in other countries.

David Dickson

Chevènement wins control of science

France's civil science is now almost totally in the hands of one man, Jeane-Pierre Chevènement, the left-wing minister of state for research and technology (see *Nature* 2 July, p.3).

Chevènement has been battling for weeks with other ministers — notably two successive ministers of industry — for the power he believes he needs to direct France's scientific future. Now he has won.

A decree published last week gives him control of the budget for all government-funded civil research, development and technology. The budgets of all public bodies concerned with such matters will henceforth be ascribed to his ministry, giving him around FF20,000 million (£2,000 million) to play with each year.

Nevertheless, the formula which gives Chevènement these powers is complicated, and is likely to be read very closely by all concerned.

Chevènement will, however, have total authority over the Centre National de la Recherche Scientifique (CNRS), which plays the leading role in supporting basic science in France. CNRS has 1,200 laboratories (including a third of all university laboratories) most of them managed jointly with the universities or other institutions but 250 of them completely independent. CNRS is a dominating influence in basic research in France, with the possible exception of mathematics; it has a staff of 25,000, of whom 8,000 are researchers.

Previously CNRS was under the authority of the ministry of universities,

and some now think that the separation from that ministry may complicate the management of joint laboratories: Chevènement is primarily interested in science as the driving force of industrial development, which may not match the views of some university departments. But he has also described science as an essential element of "culture" (music to the ears of President François Mitterrand) and wishes further to improve the international standing of French science.

Other bodies over which Chevènement will have total authority are the Délégation Générale à la Recherche Scientifique et Technique, which will act as his secretariat; the Agence Nationale pour la Valorisation de la Recherche (aimed at turning government-sponsored research into industrial innovations, through venture-capital grants); and two ministerial services, the Mission Interministérielle pour le Développement de l'Information Scientifique et Technique, and the Délégation à l'Innovation et à la Technologie.

Over other organizations, such as the Commissariat à l'Energie Atomique and the Centre National d'Etudes Spatiales, Chevènement will have control through the indirect (but powerful) lever of the budget; and also, if the relevant paragraph in the decree is so interpreted, through appointments.

This paragraph gives Chevènement the task of undertaking "all reform" of public bodies concerned with research, including "all measures have an impact on the

politics of scientific employment" — which he must countersign.

Chevènement will also "be associated with" France's efforts in international scientific cooperation, in cooperation with the foreign minister M. Claude Cheysson.

The decree outlining Chevènement's powers has required negotiation at the highest possible level, and has been signed by President Mitterrand, Prime Minister Pierre Mauroy, the foreign minister and ministers of industry and education.

The national colloquium on science and technology will now take place on 13–16 January 1982, and Chevènement is laying great emphasis on its role in defining a new politics of science in France, and the major "loi-programme" for science which he is to put before parliament next year. The colloquium is to have six principal sessions: on the cultural contribution of science and scientific responsibility; on the internal division of money for science (scientific and technical options, big science and so on); the role of science in helping France to climb out of the recession and create employment; the management of scientists (contracts of employment and so on); the role of other bodies influencing science (big industry, for example); and the political and structural means adopted by Chevènement himself. **Robert Walgate**

No rapprochement

The usually covertly political nature of education in France came out into the open last week with the sacking of 14 of the 28 regional education officials.

These officials administer education from primary to university level within the "Académies", territories which encompass several of the French departments; ever since General de Gaulle revised their powers in 1967, they have become increasingly political figures. Madame Saunier Seité, Giscard d'Estaing's minister of universities, was previously head of an *Académie*; and others became junior ministers or ministerial advisers.

In effect, this was inevitable under de Gaulle's ordinance, which loosened their hold on power and so made them more reliant on the goodwill of the government. Now fourteen unlucky incumbents have been found to be tarred too heavily with the brush of the previous administration, and must go.

This will make room for a "profound reform", the minister of education, M. Alain Savary, said last week in a press statement. The new administrators must not make politics, he said; they must obey the politics of the government. Their predecessors, by contrast, had been active against the new government both before and after the election. Some had stood as candidates for opposition parties. The deposed director of the Paris *Académie*, for example, had been Saunier Seité's chief adviser. **Robert Walgate**

Alternative energy conference

Realism the theme

Washington

At least five heads of state, including Canadian Prime Minister Pierre Trudeau and Mrs Indira Gandhi of India, will be among the participants at the United Nations Conference on New and Renewable Sources of Energy (UNERG) which starts in Nairobi next Monday. Their presence, together with energy and other ministers of industrialized and developing nations, is held by the conference organizers to indicate a measure of success in getting across their view of the political importance of alternatives to oil, coal, gas and nuclear energy.

But political weight alone will not guarantee a successful conference. UNERG is likely to set a very different tone from the large UN conferences of the 1970s, when global issues such as environmental pollution and the "human habitat" were confronted in a spirit of optimistic idealism.

From the beginning, UNERG has been organized with a more pragmatic outlook. It was, for example, agreed by a narrow vote of the UN General Assembly that nuclear power would not be included on the "new energy technologies" agenda, a move supported by many industrialized nations which feared the result would be too difficult to handle, but opposed by developing nations on the grounds that its exclusion — together with any explicit reference to conventional energy sources — would inevitably skew any results. But perhaps the main stimulus for pragmatism is the general feeling that grandiose schemes for new international bodies (such as the UN Environmental Programme, set up after the Stockholm conference in 1972), or even for a commitment to significant increases in development aid funds, are unlikely to gain support in an international mood of austerity.

In such a context, the tangible achievements of the Nairobi meeting will inevitably be limited. But Secretary General Enrique Iglesias, seconded to direct conference preparations from his permanent position as head of the Economic Commission for Latin America, remains confident that it can still have a real impact by bestowing legitimacy on energy sources frequently omitted from development planning.

One of the most practical parts of the conference has already been completed — a series of technical reports on fourteen different types of new and renewable energy sources, from wind energy to draught animals. The quality of the reports is mixed; but some, such as that produced by an international panel on wind energy, have met with wide approval. And the preparation of national contributions to the conference is said to have catalysed thinking about alternative forms of energy

production and energy planning in general — particularly in some of the developing countries — that had previously been virtually non-existent.

How these will evolve into practical initiatives remains to be seen. Several industrialized countries are said to be keen to support the setting up of research and training institutes for the various energy sources, wood-fuel being the most frequently quoted example. And the Society for International Development is promoting the idea of an international network of energy research institutes along the lines of the agricultural research network run by the World Bank.

The political debate will inevitably focus on the two factors which tend to dominate all international meetings of this nature: money (development aid contributions, primarily from the industrialized nations), and power (the organization of the UN bureaucracy).

Initially the developed and developing nations will be less far apart than at the UN Conference on Science and Technology for Development (UNCSTD) in Vienna in August 1979, where the Group of 77 arrived at the bargaining table with a proposal for a new fund to support Third World research with a budget of \$2,000 to \$4,000 million. This time the developing countries have accepted that such proposals are unrealistic. Any extra support for development of new energy technologies is therefore likely to come from changes in existing arrangements.

The conference organizers originally hoped that the meeting would coincide with the creation of a new "energy affiliate" by the World Bank, a proposal put forward by the bank's then President Robert McNamara last year as a device for raising capital for energy production schemes. So far, though, this has been vetoed by the Reagan Administration on the grounds that investment should, where possible, be left to the private sector.

With the World Bank's initiative stalled — and general agreement that the Nairobi conference should not try to set up a new energy fund — one of the most likely outcomes is an agreement that energy projects should receive a set proportion of the funds raised through a new "financing system" for science and technology, which has been in the planning stages since the Vienna meeting two years ago.

If approved in principle by the General Assembly later this year, the "financing system" would probably take over responsibility for projects at present financed through a two-year interim fund, also established at Vienna, operated by the UN Development Programme. The interim fund already supports several energy projects, such as research into the use of wind power in Mauritius and the introduction of more efficient wood-stoves into the Sahel region of Africa.

To a large extent, however, the most significant meetings will not take place in

Nairobi at all, but will be those surrounding the summit meeting planned for Mexico in October under the auspices of the Brandt Commission. This meeting, which will be attended by President Reagan, is expected to set the tone for negotiations between developed and developing nations for the first half of the 1980s; as a result, it will provide the setting within which any results from Nairobi will inevitably be judged.

David Dickson

To the gulags

The last two members of the Moscow "Working Commission to Investigate the Use of Psychiatry for Political Purposes", Irina Grivnina and Feliks Serebrov, were last month tried and convicted on charges of anti-Soviet activity. Ms Grivnina was sentenced to five years' internal exile, Mr Serebrov to four years in a labour camp plus five years internal exile.

The "Working Commission" was established in 1977 as part of the general human rights monitoring movement in the Soviet Union, and its members made determined efforts to visit "patients" confined in psychiatric hospitals because of their political beliefs. The commission produced a *samizdat* (information bulletin) giving details of its findings, and where possible provided colleagues abroad, on a confidential basis, with case notes of the patients investigated. In most cases these notes showed that by non-Soviet standards there were no grounds for compulsory hospitalization.

For these activities commission members have either been forced to emigrate (like Dr Volshanivich) or arrested and charged with disseminating anti-Soviet slander (Article 190/1), or with anti-Soviet propaganda and agitation (Article 70/1). According to Soviet judicial theory, as reiterated recently by Evgenii Smolentsev, Deputy Chairman of the USSR Supreme Court, "in the practice of Soviet courts, there are not and there cannot be convictions for religious and political beliefs" and hence any claim that there are prisoners of conscience in the Soviet Union is anti-Soviet propaganda.

According to TASS, Mr Serebrov pleaded guilty under Article 70/1 and admitted knowing that the documents he helped to prepare would be distributed in the West. TASS further recorded that he had "repented" of these actions, but some Moscow sources deny this "repentance", saying that in such a case a far lighter sentence would be expected.

The British Medical Association has tabled a motion for the September meeting of the World Medical Association in Lisbon, condemning both the use of psychiatric methods for political repression and the suppression of the "Moscow Working Commission".

Vera Rich

UK engineering council

New lambs for old

A new body to safeguard the quality of British engineers is to be set up by Royal Charter, the government announced last week. The Engineering Council, as the body will be known, is the culmination of eighteen months of heated debate since the Committee of Inquiry into the Engineering Profession, chaired by Sir Monty Finniston, recommended that there should be a new organization to oversee the education and registration of engineers.

The Engineering Council's first part-time, unpaid chairman is Sir Kenneth Corfield, chairman of Standard Telephones and Cables Limited. The Department of Industry, which is putting up £1 million to get the council started, will appoint 15-24 board members — for which it has already received 300 suggestions — and a permanent secretariat in the autumn. After a three-year transition period, the council will elect its own members and will be expected to be self-financing mainly from fees charged for registration, its chief business.



Corfield; engineering's chartered chief

The council is a disappointment to many. Some consider it a poor substitute for the statutory Engineering Authority that Sir Monty's committee had asked for. One issue in the debate has been the relationship between a new body and engineering institutions, especially the Council of Engineering Institutions, which have traditionally chartered engineers and promoted their separate interests through their own Royal Charters. Sir Monty and his supporters fear that the new Royal Charter gives the institutions too much influence. Certainly the new council will not have funds for the improvement of engineering education or even the support of students, as Finniston had asked.

The charter lays down two main roles for the council, each of which it is empowered to delegate in part to the engineering institutions: to determine standards and criteria for the education, training and experience of engineers and to keep a

register of those meeting the criteria. Engineers will be eligible to apply for three categories of registration; as professional engineers, technician engineers or engineering technicians. Registration for each category will be in three stages, the first after completing an approved course, the second after training and the third after work experience. Provision is also made for those entering the profession through unorthodox routes. And all those now registered through the Engineers' Registration Board of the Council of Engineering Institutions will automatically be registered with the new council at stage three.

The role of the Engineering Council as registering authority calls into question the future of the Council of Engineering Institutions (CEI). That body, however, expects to carry on with business as usual for perhaps two years until the new council is operating fully. Any change in its status will then mean revoking its own Royal Charter, a move which cannot be taken without a two-thirds majority among its members. The battle for responsibilities could continue much against the wishes of Sir Monty's committee and others who hoped to break the CEI's grip.

Judy Redfearn

University Grants Committee

Biting the bullet

The University Grants Committee considered earlier this year whether it should resign rather than administer the British government's 8.5 per cent cut in support for the universities, according to Dr Edward Parkes, the committee's chairman, in evidence to the House of Commons Select Committee on Education. But, in the end, the committee decided to soldier on, not wishing to be a "fair-weather committee" and believing itself to be the only group with a sufficiently detailed knowledge of the British university system.

Some Members of Parliament were clearly disappointed that the committee had not given them the tangible weapon against the government that a mass resignation would have provided. The select committee was also chagrined that Dr Parkes declined to hand over copies of his correspondence with the Secretary of State for Education, Mr Mark Carlisle, in which — according to his evidence — he had warned the British government of too rapid a contraction of the university system.

Although Dr Parkes's evidence, like that of a delegation from the Committee of Vice-Chancellors and Principals, was taken in private, a transcript of the proceedings was made public last week after the witnesses had confessed themselves puzzled that the hearing had been held in private. Perhaps the select committee had been hoping that its witnesses would have been more open, even gossipy.

Much of Dr Parkes's evidence concerned the criteria his committee had used for allocating funds to the various universities. The relatively favourable treatment for science is apparently accounted for by the "swing back to science" evident in recent applications from would-be students. Within biology, Dr Parkes said that the committee had taken the opportunity to encourage courses and departments with economic potential. Departments of social science, whose student numbers are to be cut collectively by 12 per cent in the next few years, will suffer chiefly at the "soft end", partly on the grounds that student demand is falling and partly because staff-student ratios in some of the departments concerned are too small for good research to be feasible. The grants committee had not, however, been influenced to any substantial extent by "manpower considerations".

Nor, according to Dr Parkes, had regional considerations played a part in his committee's planning except in special circumstances — the argument, for example, that the Universities of Glasgow and Strathclyde should between them be able to provide a full range of courses in higher education to satisfy the needs of students from south-west Scotland, where regional loyalties are strong.

The qualifications of students entering universities had counted in the grants committee's calculations, but Dr Parkes said last week that the committee had tried to strike a balance between institutions with high-quality entering students and those apparently able to provide less highly qualified students with good degrees and useful adult careers.

On the contentious issue of the research records of various departments, Dr Parkes rejected the criticism that too much attention had been given to the success with which departments were able to recruit grant support from the publicly funded research councils. Instead, he argued that industrially supported research usually provided university departments with a measure of overhead support, with the result that the grants committee could legitimately confine itself to the public provision of research support.

The joker in Dr Parkes's pack appears to be the quality of teaching in individual departments and universities, which was also one of his committee's criteria. Insisting that judgements of this kind must necessarily be to some extent subjective, and that little could be done to develop objective criteria based on graduation results, he will not have stilled the charge of prejudice made in the past few weeks by several universities.

Dr Parkes agreed, however, with the vice-chancellors that his committee's ignorance of the other sector of British higher education, represented principally by the 26 polytechnics, is a serious obstacle to sensible planning.

US university research Industry to provide

Washington

Increased incentives to private companies which support basic research in US universities were contained in a tax package, backed by President Reagan, which was passed by Congress on Monday this week.

The tax incentives were considerably broader than the Administration had initially proposed and unlike that part of the bill dealing with cuts in personal taxation, credits for spending on research received wide bi-partisan support.

Initially the Administration, in its tax package put to Congress in March, had concentrated on shortening the period in which research and development equipment could be written off. Both House and Senate have now passed bills under which such equipment can be fully depreciated over three years, two years less than is allowed for other capital equipment.

The protracted debate over these proposals gave individual congressmen a chance to add their own suggestions for reducing the tax burden on the private sector. One of these is the idea, originally raised by Representative Charles Vanik, that companies should receive substantial credit for money used to support basic research in universities.

When the tax bill was passed to the House Ways and Means Committee, groups such as the Association of American Universities and the American Council on Education argued that such an addition would help offset reduced federal support for university research.

Dr Donald Kennedy, president of Stanford University, told the oversight subcommittee of the House Science and Technology Committee in June that the additional tax credits, which had been put forward in a separate bill sponsored by Congressman James Shannon, would "significantly invigorate" the relationship between industry and universities "without some of the hazards that I see in the present helter-skelter pattern of affiliation".

In the same vein Dr Paul Gray, president of Massachusetts Institute of Technology, told the Ways and Means Committee that tax credits would create an "urgently needed" increase in the flow of corporate funds for university research. At present about \$210 million — or 3.5 per cent — of university research funds is provided by the private sector, the bulk of the rest coming from federal government.

Responding to such arguments, the committee not only included a tax credit for any increased research expenditures, but confirmed that existing tax provisions affecting both gifts direct to academic institutions and to third-party tax-exempt organizations, such as foundations, would persist.

However Dr Kennedy and Dr Gray were

Australasian fellowships

The council of the Royal Society has set up a new research fellowship designed to further the United Kingdom's scientific collaboration with Australia and New Zealand. Main purpose of the fellowship is to improve access to major "facilities" such as unique features of the geological, oceanographic or biological environment, astronomical centres and nuclear physics and biotechnology laboratories.

The scheme is aimed at British and Australasian postdoctoral scientists wishing to undertake research or to learn new techniques. The fellowships last from three to twelve months, and are the result of discussions between the Royal Society, its Australian and New Zealand equivalents, the UK Science and Engineering Research Council and Australasian government departments. The Australasians have yet to find the funds for the scheme, so the Royal Society, encouraged by £15,000 from BP, has forked out £50,000 for the first year of the enterprise. Interested scientists and engineers, or their potential hosts, are invited to apply.

Philip Campbell

unable to convince Mr Donald Regan, Secretary of the Treasury, that the extra credits for support of university research should be included in the bill.

The bill was eventually defeated after a bitter fight over the timing of cuts in personal taxes on the floor of the House. But the Republican-sponsored measure which replaced it, with the support of several conservative Democrats, adopted virtually identical language in its section on tax credits for research expenditures within the private sector.

There were some differences. The House bill, for example, includes corporate expenditure for university research in the base from which the additional expenditures, which would be eligible for the tax credit, are calculated. This will substantially reduce the size of the credits over those supported by the committee. In addition, the House bill specifically excludes tax credits for the support of research in the social sciences and the humanities.

The result is that there will be a small credit for basic research as well as a provision that research equipment given to a university, provided it is less than two years old, can be counted by a company as a charitable contribution.

The major question now is whether all the proposals made in the House bill will be agreed by the Senate. At present, however, the Senate version does not include the tax break for basic support in universities. Representatives from the two legislative bodies met this week to iron out their differences; in the absence of significant opposition the university tax credits survived into the final bill.

David Dickson

Brazilian research funding

Sliding backwards

Rio de Janeiro

There is uncertainty and alarm among Brazilian scientists following a series of forums held during the past few months at the request of the Brazilian Association for the Advancement of Science and involving the heads of the various funding agencies. Still no official government policy has been announced, and scientists are very much in the dark about the future of Brazil's science funding.

With inflation at 110 per cent this year and confronted with shrinking research budgets and graduate scholarships, research scientists, particularly those at universities, are concerned by suggestions made by Gerson Ferreira Filho, new director of the Financing Agency for Projects and Studies (FINEP), that his agency would abandon its policy of financing all institutions and only approve funding for specific projects. Support for institutional funding, he said, would be transferred to the Ministry of Education.

The Minister of Education, General Rubem Ludwig, in his turn, has hinted at the possibility of changing the status of 19 federal universities to that of "foundations", capable of "more financial flexibility". This would force the universities to balance their budgets by charging high tuition fees, making Brazil's education system even more discriminatory than it is now. Even with 65 per cent of the Ministry of Education's budget going to higher education, academics point out that the amount is ridiculously small. Education's share of the national budget has fallen from eleven per cent 10 years ago to less than five per cent today. And the Secretary for Higher Education, although admitting at one of the forums that "we've reached the last line of defence at the bottom of the financial pit", has made no proposals for improving the situation in the immediate future.

Another area of concern is the size of the support provided by FINEP, which amounts to \$100 million per year for all fields of science. In principle other funds are available from FINEP for industrial development projects, but these are in the form of loans to private industry and government agencies, rather than academic institutions. Funds are allocated without external review, and this lack of participation of the scientific community was strongly criticized recently in a document signed by fourteen scientific associations.

The problems of inflation have hit particularly hard at those receiving grants and scholarships from the National Centre for Research (CNPq). The recent introduction of fixed ceilings for scholarships was strongly denounced at the forums, and CNPq, with a meagre \$10 million budget, has announced a policy of

increasing the number of scholarships on offer by reducing the size of each one. CNPq's deputy director has agreed to increase the value of grants already awarded to keep them in line with inflation. Fixed ceilings, however, would apply for all new grants and there are rumours that CNPq's own budget will not be adjusted for inflation next year.

When, at one of the forums Senhor Ferreira claimed that this year's budget was 38 per cent higher than in 1980, he was interrupted by one of Brazil's leading economists, Professor Maria da Conceição Tavares, who demanded "honesty in words", saying that because of inflation a 38 per cent absolute increase implied an effective cut of 60 per cent. Suggestions from administrators that scientists and professional organizations should lobby the government for more funds were quickly rejected by the scientists present, who pointed out that lobbying requires an effective Congress. In Brazil, however, Congress does not even legislate over budgetary matters — as these have been the sole responsibility of the Presidency since 1967.

Maurice Bazin

Soviet plate tectonics

Open approval

Moscow radio has denied Soviet prejudice against the "new global tectonics". On the contrary, according to Moscow radio's world service, there is a "healthy climate" of "perfectly normal scientific controversy" about continental drift and plate tectonics. The radio's science correspondent, Boris Belitskii, said that Western speculations that there are political overtones to the controversy are "quite absurd".

As evidence, Belitskii cited two tributes which appeared in Soviet learned journals last year on the centenary of the birth of Alfred Wegener. One article, said Belitskii, was written by a strong opponent of Wegener's views, Dr Evgenii Milanovskii, while the other was written by an enthusiastic supporter, Dr Portnov, thereby making nonsense of Western "mischief-making insinuations".

Nevertheless, Belitskii acknowledged that many Soviet geologists do not support plate tectonics, considering that the theory cannot account for many aspects of continental structure and evolution.

Whether this leads to bias, or a stifling of opinion, is not clear. Some who know the Soviet Union say that discussion among geologists is now quite free. At the same time, geophysical articles in the general and semi-popular media tended, until recently, not to support plate tectonics. One of the first approving popular articles was a progress report on the Baikal-Amur Mainline railway which observed in passing that, owing to continental drift, the line, when completed, would be some 50–70 cm longer than originally planned. **Vera Rich**

Chinese university development

Help from afar

Washington

The World Bank and its soft-loan subsidiary, the International Development Association, have agreed to lend \$200 million to the People's Republic of China to support the country's efforts in meeting its present shortage of trained scientists and engineers. The loan is the first to have been made to the republic since the country assumed China's representation at the World Bank from Taiwan last May and is the biggest loan ever made specifically for building up a nation's scientific and technological base.

Half of the money will be provided by the World Bank at its standard interest rate of 9.6 per cent a year over 20 years, and the other half comes interest-free over 50 years from the International Development Association. The loans will go directly to supporting China's University Development Project, which includes among its aims an increase in the enrolment of science and engineering students at 26 leading universities from 92,000 to 125,000.

In addition, the money will be used to introduce graduate degree programme, to improve the general quality of teaching and research, and to strengthen the management of universities and the Ministry of Education.

According to World Bank officials, the government of the People's Republic of China will contribute an extra \$95 million to the University Development Project as part of a general effort to increase undergraduate enrolment by 7 per cent a year up to 2.2 million in 1990. Graduate programmes will raise their enrolments from virtually zero to 200,000 by 1990.

Of the total amount of \$295 million for which the project has at present budgeted, about \$160 million will be used to buy instructional and research equipment. Most of this will be obtained through international competitive bidding, a standard requirement of World Bank loans. Preference will be given to local manufacturers, but World Bank officials admit that "the amount of equipment contracts to be awarded to local suppliers through international competitive bidding is expected to be small".

China's determination to proceed with its plans for science and technology education contrast with its decision substantially to reduce previous commitments to the purchase of capital research equipment. Plans for obtaining both a particle accelerator — which was to have been built to designs produced by the US Department of Energy — and a telecommunications satellite which was to have been provided through the National Aeronautics and Space Administration, have both been shelved because of the lack of available capital.

David Dickson

CORRESPONDENCE

Creation "copout"

SIR — In a critique of American Creationism (*Nature* 2 July, p.95), Darnbrough, Goddard and Stevely state "no plausible theoretical model exists which provides a mechanism for the spontaneous generation of nucleic acids as informational macromolecules specifying polypeptides which themselves mediate the replication and expression of that information". This statement is much like the definition of the same scientific chicken-egg problem by J. Monod (1971) and K. Popper (1974).

One can argue plausibility indefinitely because the assessment of that quality is highly subjective. However, there has appeared, through *experimental* demonstration, a laboratory model of a cell-like structure with many of the properties required. This structure is composed of lysine-rich and acidic thermal polyamino acids¹ that complex with each other to form cell-like structures. Both in solution and in suspensions of particles, these (*self-ordered*) polyamino acids catalyse simultaneously the formation of peptides and polynucleotides from ATP and free amino acids (see ref.2). This model so far leaves unanswered many of the questions about the origin of the genetic code, but it demonstrates that the question is not as scientifically imponderable as Popper and Darnbrough *et al.* have suggested. It does provide answers to some questions, such as how the two kinds of macromolecular synthesis could first have been closely coordinated. The sequence theoretically derived from those experiments is: amino acid sets → protocells → nucleic acid + protein

I would not criticize Darnbrough *et al.* for not being aware of advances as recent as these (further details in the press). However, to attack an area of science because it has *not yet* reached a given stage, and then to argue a need for resorting to supernatural explanations because specific scientific answers are not reported, consolidated or agreed upon, is what is referred to in an American idiom as a "copout".

SIDNEY W. FOX

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1. Fox, S. *Nature* 205, 328-340 (1965).

2. Fox, S. & Nakashima *Bio Systems* 12, 155-156 (1980).

Attack on Tamuz

SIR — The editorial entitled "Making Israel Atone for Tamuz" (*Nature* 18 June, p.523) has shocked and dismayed many of your faithful readers. It appears that *Nature*, a journal that has hitherto enjoyed an unparalleled reputation in the scientific community for publishing innovative and careful scientific observations, is now embarking on a new course as a "yellow sheet" of political comment. One can only fear that subjective, biased, political diatribe as represented by your editorial will sully and finally displace the elegant work that has heretofore been the mark of publications in *Nature*.

I must admit that the editorial was cleverly written. Under the guise of a great concern for the reputation of the Non-Proliferation Treaty the editorial proceeds to mount a political attack on Israel and depicts Iraq, a signatory of the treaty, as an innocent, wholesome and wronged party, even deserving financial redress through "international legal processes" for the destruction of its means for producing awesome weapons.

Scientists are wont to deal with observations and to derive probability statements for predicting future phenomena based on past observations. Let us apply some logic to the editorial at hand. Unfortunately, although Iraq is a signatory of the Non-Proliferation Treaty, past actions have shown that in the case of Iraq "the sword is mightier than the pen". In the course of history the signatures of dictatorial governments have proved worthless. Iraq, by its own declaration, has maintained a continued state of war with Israel and has recently branched out in its military adventurism by an unprovoked attack against its neighbour, Iran. Iraq has been at the forefront of frenzied calls for the total destruction of Israel and has given both financial and military support to the terrorists who have revelled in the wanton murder of innocent civilians, especially women and children. Parenthetically, I did not note editorials in *Nature* either denouncing the bombing attack by Iran, albeit unsuccessful, against the nuclear reactor in Iraq, or decrying the slaughter of innocent women and children in Israel by terrorist attackers.

The editorial does not at all address the question as to why Iraq was stockpiling uranium suitable for the manufacture of atomic weapons. Much faith is placed in international commissions and the cursory inspections of the Iraqi reactor. It is ludicrous to believe that a government bent on production of nuclear weapons could not hide such facilities. Furthermore, what good is the knowledge that nuclear weapons are being produced once all the production capabilities, including the raw materials, are in place? Would a contrite editorial in *Nature* bring back to life the many thousands of casualties that would result from even one atom bomb dropped on Israel? Or might the response to such an unthinkable event be equivalent to the world response witnessed during the Nazi outrages? Even the United States government was quite concerned by Iraqi intentions as evidenced by the testimony of Mr Roger Richter before the US Senate Foreign Relations Committee.

As a native-born American, and a former officer in one of the US uniformed services, I am offended by the ugly term "Zionist vote". Fortunately, the majority of American voters speak to reality, and feel an affinity for Israel as the bastion of an open and democratic society in an area where tyrants and dictators prevail.

Unfortunately, the threat of nuclear retaliation is perhaps the only deterrent that Israel has against total annihilation by its truculent neighbours. Indeed, it appears that not only does Israel require this deterrent against its threatening neighbours, but perhaps also against such editorials as that in *Nature*.

Finally, aside from the question as to

whether one does or does not justify the Israeli destruction of the Iraqi nuclear reactor, I believe that one can assert that editorials of political diatribe do not belong in a premier scientific journal such as *Nature*.

S.Z. HIRSCHMAN

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Certain points raised in this letter have been discussed in a subsequent editorial (*Nature* 16 July, p.185) — Editor, *Nature*.

Researchers insecure

SIR — "Poaching" of ideas and staff between competing institutions is not a new phenomenon (A.J.S. Davies, *Nature* 2 July, p.96), though I doubt if it has (yet) reached serious proportions. A more serious problem, exacerbated in recent years by economic constraints, is that research teams usually include several young postgraduate and postdoctoral workers financed by short-term grants of 1-3 years' duration. The present system by which these are made available does not permit, let alone encourage, such scientists to remain in the teams where they begin to develop their expertise. There are two powerful reasons in particular for this. One is the present dire shortage of permanent posts in the academic sphere. The second is that it now appears to be the general policy that a young scientist should not be the recipient of more than 2 three-year grants from such bodies as the Medical Research Council. Consequently, after 6 years, such people may be obliged to find other employment anyway.

In most cases, the prime motivating force for a young scientist is not so much the chance to earn a large salary as reasonable security in order to develop his or her own skills and ideas over the long term. To be in a constant state of anxiety for the future, no matter what one achieves, seriously undermines one's ability to do this. Sadly, neither the universities nor the major funding bodies (the government and research councils) in Britain seem to have grasped this simple concept.

A certain degree of flexibility for movement between institutions is highly desirable, but many young scientists would welcome a contract that bound not only them but also their employer in the long term. Present conditions in Britain promote academic paralysis by destroying flexibility — those with secure jobs stick to them — while denying encouragement and opportunity to the young, and with that the long-term success and well-being of research teams.

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Erratum

In a letter published in *Nature* 14 May, p.104, the name of one of the members of the (US) Xeroderma Pigmentosum National Registry was omitted. The full list of members is: Alan D. Andrews, James L. German III, Kenneth H. Kraemer and W. Clark Lambert.

NEWS AND VIEWS

Towards a total human protein map

from Brian F.C. Clark

Now that such powerful techniques are available for the sequencing of DNA and the study of genome organization, some scientists are turning to what might be the next large-scale challenge — the separation, identification and measurement of the gene products.

At a recent workshop*, the possibility of making a complete catalogue of animal cell proteins using two-dimensional gel electrophoresis was discussed. In man there are at least 30,000 different proteins (although maybe only 10,000 in a given cell type) and the technique has already been used to separate and catalogue more than 1,200 of them (R. Bravo *et al. Cell Biol. int. Rep.* 5;93, 1981).

At the workshop it was confidently stated that it will be possible to detect 2,000 to 3,000 proteins with present technology. Further progress can be made by fractionating cellular components before carrying out two-dimensional separation. This will bring many proteins present in small amounts to the level at which they can be detected.

There are three main problems in trying to produce a catalogue of proteins for human or any other animal cells: how to make methods of protein identification reliable and reproducible; how to store the immense amount of data produced in a usable form; and how to name and catalogue the separated proteins.

The question of reproducibility is clearly the most serious problem and needs to be solved first. At present gel chemistry is not under complete control and, among the many parameters discussed, it was generally agreed that the first essential is the standardization of the commercial production of ampholytes for the first separation by isoelectric focusing. Attempts have been made to overcome standardization problems by R. Bravo (University of Aarhus), L. Anderson (Argonne National Laboratory) and J. Garrels (Cold Spring Harbor Laboratory) and a representative of a commercial concern expressed confidence that the requirements of the research scientists could be met in the future. Indeed, an

arrangement was to be made to provide specified laboratories with samples of ampholytes until all appropriate criteria were met. Since future automatic measurements and clinical applications depend on the reproducibility of the technique, this arrangement was of great significance for the workshop.

In the two-dimensional separations it is now usual to take a total cellular protein extract which can be radioactively labelled, denature it, and apply it to two different first dimensions. Isoelectric focusing is used to separate neutral and acidic proteins and non-equilibrium pH gradient electrophoresis for the basic proteins. After the first two dimensions have been run, each separated sample is applied to an identical polyacrylamide gel containing SDS to provide the second dimension of electrophoretic separation.

J. Garrels' laboratory routinely standardizes total protein extracts by using a number of different concentrations of polyacrylamide and several pH ranges. In a simpler, two-dimensional standardization (in cases for which it is justifiable to lose some basic protein resolution) his group runs only a broad-range (pH 3.5 – 10) isoelectric focusing electrophoresis for the first dimension.

After separation the protein locations on the gel have to be visualized. Discussion was rather brief but there was a general consensus that fluorography provided a better and more accurate method than autoradiography. With unlabelled proteins the recently used silver stains are much more sensitive than Coomassie blue but produce variable results with gels more than one millimetre thick.

The problems of data handling are obviously immense — even at the present time more than 1,300 proteins may be recorded in different cell types and under different conditions, and L. Anderson reported that his laboratory has already run 40,000 separations. There was, however, much optimism about current progress. Already it seems that automatic measurements of protein locations, together with computer storage and retrieval, will be possible in the laboratories of N. and L. Anderson, J. Garrels, K. Lonberg-Holm (Dupont), L.E. Lipkin and P.F. Lemkin (NIH) and E.P. Geiduschek (University of California, San Diego)

within the next year. Since these laboratories have access to medium-sized computer facilities, it was reassuring to small laboratories that A. Freiburghaus (University of Cambridge) was also optimistic of finishing his plans for general automatic measurement of two-dimensional gels using more standard university facilities.

There was general agreement on the best approach to the third problem, that of the naming and cataloguing of the proteins. It was felt that, at present, any system relying on accurate positional information was doomed to failure and that it is far too early to try to set up a master nomenclature. Estimates of when this might be possible varied from three to five years. With this problem in mind, the Aarhus group described the advantages of labelling small numbers of cells with ^{14}C -labelled amino acids so that raw data may also be preserved for long periods.

Different nomenclatures will probably be necessary for different cell types. It was agreed that the current different nomenclature systems used in different laboratories should continue but with the intention of switching to a master plan when the separations can be suitably reproduced. Meanwhile, it was agreed that laboratories involved in cataloguing cell proteins should exchange samples and marker proteins so as to be able to cross-refer to separations in different laboratories. If the cross-referencing were done, then it should be possible at future workshops to assemble the available information (tissue specificity, modifications, hormonal responses) for the 100 or so most prominent proteins and to publish a compendium of such data with representative maps. The problem of protein identity was also discussed and a general appeal was made to anyone working with purified identified proteins to allow them to be located on the standardized two-dimensional gel separations.

The workshop forecast that within a short time several data sets of catalogued proteins will be established in the USA and Europe. Possible locations would be the Argonne National Laboratory, Cold

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* The EMBO workshop on 'The standardization of a numbering system of animal cell proteins separated by two-dimensional gel electrophoresis' was held at Aarhus University, Institute of Chemistry, June 30 and July 1 1981, and was organized by J.E. Celis of the Department of Structural Biochemistry, Aarhus University.

Spring Harbor Laboratory and the European Molecular Biology Laboratory.

It was agreed that it was desirable to support a number of future research projects to obtain as complete a catalogue of proteins as possible for a small number of centrally important cell types. Probably the laboratories of L. Anderson, J. Garrels and J.E. Celis would concentrate at first on HeLa, human fibroblast, human lymphocyte and mouse fibroblast cell types. The choice of the last cell type arose from the availability of genetic data for the mouse which, in future, will allow changes in the protein map to be related to changes in genotype. In this respect J. Klose (University of Berlin) described promising results from experiments in which he identified a small number of protein changes in mouse cells treated with the mutagen methylnitrosourea.

The great potential of the method in clinical diagnosis was described by E. Jellum (University of Oslo) and L. Anderson. Jellum described the 'Janus collection', an impressive stock of human blood samples from 63,000 patients set up in Oslo in 1973. Since the patients have had samples taken yearly, when they develop a

new disease it is possible to search through their protein record for several years before the disease was detected. Clearly a catalogue of human gene products could make it possible to detect metabolic irregularities and diseases, such as cancer, which affect gene expression.

In terms of current costs, J.E. Celis's group has estimated that one two-dimensional gel analysis can be made for thirty dollars, using ^{14}C -amino acid labelling of cells. For the development of a computerized program for handling large numbers of reproducible analyses and the establishment of a human protein catalogue, the Argonne National Laboratory has estimated that a sum of the order of ten million dollars is required. The general impression given by the research workers involved was that a grant of a million dollars a year would be feasible from US granting agencies.

The meeting ended on an optimistic note with the belief that present technical problems can be solved, and plans for co-operation and for communication of unpublished data among the laboratories which are developing the technique can be implemented. □

hormone.

Mouse mammary tumour virus (MMTV) is a RNA virus that may be transmitted as a virion, producing mammary carcinomas in female mice and productive infection in rodent tissue culture cells. Like other RNA tumour viruses, it replicates by synthesizing a viral DNA copy. MMTV-like DNA is also present as an endogenous provirus in the genome of mice. In mammary tumour cells and in virus-infected cells, glucocorticoid hormones rapidly and dramatically enhance the rate of synthesis of MMTV RNA. Two recent papers from the Swiss Institute for Experimental Cancer Research describe the cloning of MMTV DNA, the introduction of that DNA into mouse tissue culture cells and the regulation of MMTV RNA expression in the cells thus transformed. In one case, the full genome of MMTV was cloned from the closed circular DNA of infected rat hepatoma cells (Buetti and Diggelman *Cell* **23**; 335, 1981). In a second approach, the MMTV provirus and flanking host cell sequences were cloned from a mouse liver DNA library (Hynes, Kennedy, Rahmsdorf and Groner *Proc. natn. Acad. Sci. U.S.A.* **78**; 2038, 1981). In each case the cloned DNA was introduced into thymidine kinase-deficient mouse L-cell fibroblasts, cells that have glucocorticoid receptors. The now-standard technique of co-transfection with DNA containing the herpes thymidine kinase-proficient cells was used to introduce the MMTV DNA. In this way, L-cell clones, each carrying multiple copies of the MMTV DNA, were obtained. Treatment of the transformed cells with the glucocorticoid dexamethasone resulted in a 5-10-fold increase in the level of poly-(A)-containing MMTV RNA. Expression of the cloned DNA was thus shown to respond to hormone in a manner similar to that found in cultured mouse mammary tumour cells or virus-infected rodent cells.

Hormonal regulation of another cloned gene is reported by Kurtz of the Cold Spring Harbor Laboratory (*Nature* **291**; 629, 1981). The rat protein $\alpha_{2\text{U}}$ globulin is synthesized in liver and excreted in urine. It is regulated *in vivo* by multiple hormones, including glucocorticoids, which enhance, and oestrogens, which suppress, its synthesis. The protein is encoded by a multi-gene family. Two of these genes were cloned and the cloned DNA introduced into L cells using the herpes thymidine kinase co-transfection technique. L-cell clones transformed with one of these genes showed dexamethasone-induced enhancement of $\alpha_{2\text{U}}$ globulin mRNA levels in 8 of 12 cases, and transformation with the other gene conferred a positive response in 4 of 9 clones. Again, individual L-cell clones each carried multiple $\alpha_{2\text{U}}$ globulin gene copies.

The question of how glucocorticoids regulate genes is but a fragment of the problem of differential gene expression, of how genes are turned off and on by differ-

Hormonal regulation of cloned genes

from Philip Coffino

How do steroid hormones work? Glucocorticoids and the sex-related steroids, oestrogens, progesterones and testosterone, have profound effects on development and differentiation. A consensus exists on the early and late steps of their action. The hormones enter a cell and bind to a specific cytoplasmic receptor. The steroid-receptor complex undergoes a structural change that results in its entry into the nucleus. In the nucleus a series of events ensue that lead to an increased rate of transcription from specific genes, with a consequent increase in the steady-state level of mRNAs generated from these transcripts. (Other mechanisms, such as changes in mRNA stability, may also play a part in some cases.) The nature of the events involved in transcriptional regulation by the steroid-receptor complex in the nucleus is obscure.

This problem is a special case of a more general one: how is gene expression controlled to produce cellular differentiation

and modulated by hormones and other physiological effectors in differentiated cells? Cloning of eukaryotic genes in recent years has yielded considerable information on gene structure, but relatively little on regulation. Cloned genes, for example globin and ovalbumin, have been placed in cells and shown to function there. However, in their new environment, these genes did not respond to the regulatory signals that work *in vivo*. Recently, a series of papers has demonstrated that hormone-dependent control of expression can take place in cloned genes put back into cells. This work and natural extensions of it promise to help elucidate the molecular mechanisms of steroid hormone action.

An attractive model, derived from our understanding of how gene expression is managed in bacteria, postulates that the steroid hormone-receptor complex binds with specificity and high affinity to a region of DNA which lies near the gene whose transcription it regulates. The model predicts, as has now been found possible in two distinct systems, that isolated DNA containing the regulable gene, introduced into a hormone-responsive cell, should show regulation dependent on the

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entiation and by physiological changes. The studies described here show that, placed together, a cell and a cloned gene can modulate the level of that gene's RNA product in response to a glucocorticoid. The nature of the contribution of each is not resolved. Does the hormone-receptor complex interact directly with sequences in or near the cloned gene, in flanking host cell sequences or at more distant sites? Do all copies of the gene function, or only some, and if the latter, what distinguishes the responsive copies? Is the regulation shown here transcriptional or does it lie at the level of RNA stability or processing? Why do the cloned genes work at all in L cells? In the case of MMTV the gene is already there in the form of a provirus, but is not expressed, while the normal functional environment of the α_{2u} globulin gene is a liver cell, not a fibroblast. Can

these differences in function be accounted for in terms of chromatin structure, DNA methylation, the nature of flanking sequences or in some other way?

It is probable that we will soon have answers to those and related questions. The MMTV α_{2u} and other hormone-responsive genes, natural or constructed, will be sequenced, cut, modified and asked to perform in cells, and the transformed cells' DNA and chromatin will be picked at with nucleases and restriction enzymes. Experiments of this kind will define the elements needed to make the system work and this understanding will guide efforts to design a fully defined hormone-regulated *in vitro* transcription system constituted of purified components. A crucial early step of this ambitious programme has been achieved and the rest will find no lack of willing hands. □

Eruption mechanics on the Earth, Moon and Mars

from G. Wadge

THE discovery of volcanic eruptions on Io by the Voyager 1 spacecraft¹ was exciting visual proof that volcanism, in this case based on sulphur, is a major process on the surface of at least one planetary body other than Earth. There is abundant, though less spectacular, visual evidence of past volcanism on Mars and the Moon; most of this volcanicity occurred in the early history of these planets. Volcanologists are faced with the problem of how to interpret this evidence in terms of eruptions which ended billions of years ago. One of the most productive methods has been to compare the known magma properties and conduit systems of volcanoes on Earth with the equivalent putative values for the other planet, deduce what volcanic landforms these eruption mechanics should produce and see whether these agree with the observations. Recently Wilson and Head² reviewed the mechanics of erupting basaltic magma on the Moon using this approach. Their conclusions, perhaps rather surprisingly, generally emphasize the similarities between terrestrial and lunar flood basalt eruptions.

On both bodies magma rises to less than 2 km below the surface before gases begin to exsolve, mainly H₂O and CO₂ on Earth, and CO on the Moon. Wilson and Head assume that CO is the main lunar volatile phase and is soluble in silicate magmas —

but there is little direct evidence. The rates of rise of magma before this vesiculation are limited by the degree of cooling and the attendant increase in viscosity, and by the yield strength of the magma. Wilson and Head find that a very small range of conduit widths (0.1–4 m) is adequate to explain the whole spectrum of terrestrial eruptions, from the magma that barely reaches the surface to gigantic eruptions exemplified by the Columbia River flood basalts. The most striking feature of lunar volcanism is the great length and volume of many of the recognizable mare flows. This study clearly shows that the feeding fissures of these huge, high-effusion rate eruptions need to have been only slightly wider than those on Earth, perhaps 10–25 m. We see little evidence of these source fissures on the Moon and should not expect to, for they are too narrow.

Lunar basaltic magmas came from deeper sources (~100 km) than most terrestrial basalts and were also relatively denser compared with the overlying crust. Buoyancy is presumed to be the main force responsible for magma rise on the Moon as it is on the Earth. Thus lunar magma pressures should have been lower and the effusion rates less than terrestrial values. This conclusion is contrary to the observed size of the lava flows. A hydrostatic solution to this problem was proposed by Solomon³ involving regional loading outside the mare basins. Wilson and Head now show that magma density contrasts were of the same order as those calculated

for Earth (a few hundred kg per m³). The large magnitude of the lunar eruptions was due to the local extensional tectonic environment around the mare which allowed particularly wide fissures to develop. Lunar effusion rates were probably not exceptionally large compared with those for terrestrial flood basalts. What is remarkable is that some of the calculated eruption volumes (10³–10⁴ km³) represent a significant fraction of the total volume of mare volcanism (~10⁷ km³).

CO concentrations in lunar basalts were probably about an order of magnitude less (a few hundred p.p.m.) than average volatile contents of terrestrial basalts. As magma rose to the surface, this relative deficiency of volatiles would be compensated by an increased release of energy per unit mass due to the decompression to zero atmospheric pressure at the surface. The resultant disruption of magma into fragments would allow wide dispersion but the lack of an atmosphere would preclude the complex differential sorting processes typical of terrestrial pyroclastic eruptions. Instead, spectacular fountains of magma clots must have blanketed large areas around lunar vents, and in many cases these clots, uncooled by atmospheric interaction, would have recombined on hitting the ground to form lava flows.

Unlike the Moon, Mars has a thin but appreciable atmosphere and a gravity field intermediate between those of the Moon and Earth. One would expect martian volcanism to be a hybrid of the characteristics displayed by its planetary neighbours. Preliminary calculations, again by Wilson and Head⁴, indicate that it should resemble Earth volcanism more than that of the Moon. They predict vigorous, convecting eruption columns, growth of gas bubbles in magma to about four times the equivalent size on Earth, and the common occurrence of high-pressure, explosive volcanic eruptions caused by magma interaction with sub-surface ice. Of course, we know less about the tectonic setting and much less about magma compositions on Mars than we do for the Moon. If these factors are not too different from the values assumed by Wilson and Head, then martian eruption mechanics should make the development of ignimbrites, by the collapse of large eruption columns, even more common than on Earth. Can we see these ignimbrites? The answer given by Greeley and Spudis in their review⁵ of the Mariner and Viking data is that despite several claims for their identification, unequivocal proof is lacking. Failure to identify ignimbrites on Mars could necessitate revision of the eruption models of Wilson and Head although it is more

G. Wadge is at the Lunar and Planetary Institute, Houston.

1. Masursky, Schaber, Soderblom & Strom *Nature* **280**, 725 (1979).
2. Wilson & Head *J. geophys. Res.* **86**, 2971 (1981).
3. Solomon *Proc. 6th Lunar Sci. Conf.* **10**, 121 (1975).
4. Wilson & Head *Abstr. 12th Lunar Sci. Conf.* **1194** (1981).
5. Greeley & Spudis *Rev. geophys. Space Phys.* **19**, 13 (1981).

probable that we need to look longer and more closely.

The calculations of Wilson and Head assume that the pressure gradients driving erupting magma are equal to the lithostatic gradients — a reasonable assumption for generalized modelling. It is clear that this does not hold for all eruptions on Earth. Repeated fracturing and deformation of

the surface during the eruption of central volcanoes is proof of this. Mars has huge central volcanoes which, like their terrestrial counterparts, exhibit a variety of evolutionary forms and undoubtedly have complex internal structures. In future we will need to incorporate elements of these massive surface structures into models of martian eruption mechanics. □

occurs in the McArthur region, which further displays redox-related mineralization in the Woollogorang Formation, some 3,000 m below the well known HYC deposit.

Further evidence against 'biogenic' lead-zinc mineralization is the presence of saddle dolomite in carbonate host rocks of many lead-zinc occurrences. The mineral indicates relatively high temperatures (up to 150°C) and is perhaps formed along with sulphides during inorganic sulphate reduction.

At Mt Gunson, copper-iron sulphide minerals hosted by laminated mudstones and dolostones are of two generations. The sulphur itself was introduced at the same time as ancient coastal sediments and then reacted with sedimentary iron to form pyrite. This was replaced by copper introduced along faults and other channelways.

C.C. von der Borch (Flinders University, South Australia) outlined how studies on modern depositional systems can lead to a better understanding of sedimentary controls on ore deposition. Noteworthy are deposits in oceanic rift zones (for example, the Red Sea and East Pacific Rise), which provide information on the sources of metal-enriched hydrothermal fluids, and contemporaneous deposition of metal sulphides, in settings analogous to some major ancient deposits. By contrast, modern shallow marine, subaerial and lacustrine sediments, while often resembling ancient counterparts that host stratiform and strata-bound ores, rarely contain metal sulphide concentrations significantly above background.

The main factor controlling metal sulphide accumulation in reducing sediments remote from hydrothermal influences appears to be the supply of reactable metals rather than of sulphide. In many present-day shallow marine sediments, and in some deep-sea sediments, bacterial sulphate reduction is of sufficient intensity to generate sulphide concentrations one to two orders of magnitude higher than those actually observed. In the Holocene paralic carbonate complexes in northeastern Spencer Gulf, South Australia, low levels of metals limit mineralization even though the hydrological regime of this environment resembles those inherent in a number of models of ore genesis.

Potential environments for large-scale biogenic sulphide deposition are those in which metal-enriched groundwaters mix with organic-rich, sulphidic sediments — perhaps exemplified by the Denali chalcopryite-pyrite deposit in Alaska. The possibility of deposits containing sulphide of both hydrothermal and biogenic origin

Sedimentary sulphides

from Philip Trudinger and Preston Cloud

THE recognition that present reserves of minerals may be nearing exhaustion has increased interest in the help that models of ore genesis might give in the search for deep-seated deposits of commercial-grade minerals. That some strata-bound base metal sulphide ores may have formed syngenetically, that is, in the early stages of sedimentation, by deposition in sedimentary pile rather than by hydrothermal replacement was first proposed in the early 1950s. Since then, syngenetic models have been proposed for many deposits and often imply low-temperature deposition with the involvement of biological activity in mineralization. A timely review of evidence for and against biological and other syngenetic processes in sulphide ore genesis was provided at a symposium* held in Australia earlier this year.

The characteristics of strata-bound sulphide ores were described by L. Gustafson (Australian National University, Canberra). The strata-bound sulphide and sediment-hosted lead-zinc and copper ores are features of the oxygenous Earth, and are no older than 2 Gyr. The deposits are in tectonically active intracratonic settings, commonly associated with volcanism, with indications of aridity or hypersalinity. The local settings are ones in which groundwater can move to shallow sites of sulphide deposition. The deposits are characteristically associated with redbeds, either beneath or interbedded with them. This is particularly true of copper ores, most of which are found in reduced sediments, often at a redox interface; for example, between red and green sediments. Zoning patterns are usually related to 'plumbing systems', both stratigraphically and areally. The timing of metal fixation

relative to deposition and diagenesis, and the temperature of mineralization, are of crucial importance but are, as yet, poorly understood. Sulphur isotope values reflect processes at the sites of deposition rather than the source of the sulphur. Finally, unlike volcanogenic deposits which range across the copper-zinc axis on a lead-zinc-copper ternary diagram, the strata-bound copper and lead-zinc deposits mostly cluster in the copper corner or line up along the lead-zinc edge respectively.

Gustafson emphasized the problems arising from variable usage of the terms syngensis, diagenesis (processes taking place after sedimentation) and epigenesis (processes taking place after sediments are compacted). Different scientists draw their boundaries at different places. He concluded that most of the deposits in question are diagenetic, with metals derived from within the sedimentary sequence but outside the areas of ore deposition. Local reduction of sulphate is probably responsible for deposition of most copper and some lead-zinc, but more than one source of sulphur and mechanism of fixation exists. Variations are, however, observed towards both later epigenetic, and earlier syngenetic, processes.

Several papers dealt with specific ancient strata-bound sulphides — especially the Mount Isa ores of north-west Queensland, the McArthur deposits of Northern Australia, and the Mt Gunson copper deposits of the Stuart Shelf, South Australia. Methods of investigation included isotope tracers, mass-transfer modelling, fluid inclusion palaeothermometry and palaeosalinity, detailed stratigraphy and petrology and biogeochemistry. A consensus of opinion favoured a penecontemporaneous origin for the lead-zinc-silver ores at Mount Isa and McArthur, compared with an epigenetic hydrothermal origin for the associated copper ores at Mount Isa. The ore metals and most of the sulphide were introduced by exhalative processes although there is evidence that some of the sulphide in pyrite was of biogenic origin. Local karst-associated mineralization also

*A symposium on *Sulphide Mineralization in Sediments: Current Status of Syngenetic Theory* was held at the Australian Academy of Science, Canberra, from March 2 to 4, 1981. It was sponsored by the Baas Becking Geobiological Laboratory and formed part of the programme of IGCP Project 157 on *Early Organic Evolution and Mineral and Energy Resources*. It was attended by over 100 scientists from the mining and petroleum industries, universities and government research laboratories. Abstracts and several full papers will be published in December 1981 in the *Bureau of Mineral Resources Journal of Australian Geology and Geophysics* 6, No. 4.

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is suggested by isotopic studies on the McArthur deposit and on the modern sediments of the rift-related Guaymas Basin, Gulf of California.

Although it is now well established that bacterial sulphate reduction is a significant and rapid biochemical process in modern anoxic sediments, caution was advised in extrapolating local rates of sulphate reduction to regional models of mineralization. Rates are affected by many factors, including abundance of organic matter, rate of flow of interstitial water, bioturbation and, for intertidal sediments, the amount of time the sediment is wetted.

Sulphur isotope data are frequently used in inferring the origins of mineral deposits and in interpreting the diagenesis of sulphur. The interpretation of such data is not, however, without difficulties. Patterns of $\delta^{34}\text{S}$ frequencies in sulphides of modern sediments can be related to the supply of sulphate (open versus closed systems) but not to geological settings. In the reducing sediments of the Bali Trough, ^{32}S in pyrite increased with depth, a trend similar to those reported for cores from the McArthur and Kupferschiefer deposits but which at present defies explanation. However, broad trends in the isotopic compositions of ancient minerals with time are perhaps more amenable to interpretation and suggest that sulphate became the major sulphur component in the hydrosphere, and bacterial sulphate reducers widespread, at about 2 Gyr BP. Anomalous $\delta^{34}\text{S}$ distributions in 2.7–2.8 Gyr old sulphides of the Michipicoten and Woman River deposits could have resulted from reduction of an oxidized species (sulphite?) other than sulphate.

Further papers dealt with the production and diagenesis of organic matter, the driving force for all biogeochemical processes, and the significance of organic carbon in ancient deposits. Benthic oxygen-evolving prokaryotes were seen as major organic carbon producers in marginal aquatic environments of arid and semiarid climates. Both field and laboratory studies indicated, however, that the bulk of the organic matter is rapidly remineralized, particularly by sulphate-reducing bacteria.

In relatively young sediments, specific organic molecules of diagenetic origin provide clues to sources of organic matter (for example, marine versus non-marine), depositional environment (oxic compared with anoxic) and maturity of organic matter. With time, carbonaceous material undergoes a complex series of reactions leading to loss of volatiles and formation of different products — kerogen, fossil fuels or graphite. Even graphite may be oxidized to CO_2 by water at above 200°C , and can be removed from the system without trace. Thus, while reduced carbon (or graphite) occurs in sediments of all ages, including the 3.8 Gyr old metasediments at Isua, south-west Greenland, its significance can prove difficult to determine. Nevertheless,

considerable progress has been made towards identifying major biogeochemical evolutionary events in Proterozoic and older history through the use of carbon isotopic data on kerogens, together with fossil and other geochemical evidence. These data are now all consistent with the view that oxygenic photosynthesis had evolved by the late Archaean and that, thereafter, first the hydrosphere and then the atmosphere were progressively oxygenated.

The symposium highlighted advances made over the past decade in our knowledge of mineral deposits and modern

depositional systems. It stressed the need for continuing multidisciplinary research on the evolutionary cycles of elements involved in the making of either sedimentary ore deposits or fossil fuels. These cycles are, or may be, affected by biological processes at some stage in their history. Whether that is called syngenetic or diagenetic seems to be more a matter of discipline and definition than of timing. To the extent that we detected consensus, it was to the effect that major sulphide deposits seem to include both post-depositional sedimentary and later hydrothermal components.

Synthesis and function of metallothioneins

from J. Kägi, T.L. Coombs, J. Overnell and M. Webb

As cadmium is a ubiquitous component of the Earth's minerals, its transfer to animals and man through food chains cannot be avoided. Since the metallic ion is cumulative, long-lived mammalian species, even in uncontaminated environments, can accumulate appreciable body burdens of Cd^{2+} during their lifetimes. Most of this Cd^{2+} is stored in the liver and kidneys as a soluble low-molecular-weight metalloprotein which contains not only appreciable amounts of Cd^{2+} and Zn^{2+} , but also an extremely high number of cysteine residues. The metalloprotein, named 'metallothionein', is a metallo-derivative of the sulphur-rich apoprotein, thionein. Thionein is an inducible protein and synthesis of usually short-lived metallothioneins, in which Zn^{2+} and Cu^+ are the major bound cations, occurs when the intakes or body burdens of these 'essential metals' are excessive.

An explosive increase in interest in these metalloproteins followed observations in the 1960s that they accumulate in the liver and kidneys of rabbits in response to administration of cadmium salts. As there was evidence from Japan of a relationship between cadmium pollution and the Itai Itai disease, the hypothesis was put forward that the inducible synthesis of metallothionein formed a defence mechanism. Evidence soon accumulated that besides participating in cadmium sequestration, metallothioneins play a fundamental part in the homeostasis of certain essential cations, principally Zn^{2+} and Cu^{2+} , a function undoubtedly dependent upon their primary, secondary and tertiary structures. Analysis of recent

developments in the chemistry of metallothioneins, with its implications in biochemistry, nutrition, toxicology and medicine, formed the main objective of a recent meeting*.

Sequencing and physico-chemical studies (J.H.R. Kägi, University of Zurich) show how the primary and tertiary structures have been conserved during many evolutionary stages. From UV, circular dichroism and magnetic circular dichroism spectroscopic studies on experimentally prepared cobalt(II)- and nickel(II)-metallothioneins, as well as the natural zinc- and cadmium-proteins, M. Vasak (University of Zurich) concluded that the cysteine residues are bound by their thiolate groups to these metals in a tetrahedral configuration. Electron paramagnetic resonance and magnetic susceptibility studies of the cobalt derivative suggest that the metal sites are joined in clusters with some of the cysteinyl residues serving as bridging ligands between the metals, as exemplified by an adamantane-type structure.

Mammalian copper-metallothionein and the potential biological significance of its inducibility in some tissues by copper administration were discussed by I. Bremner (Rowett Institute, Aberdeen). The binding of copper to metallothionein differs from that of other metals and the metal to sulphur stoichiometry is much lower. The copper of anaerobically prepared metallothioneins is bound in the cuprous state in the form of Cu(I) -thiolate complexes. The binding mode is consistent with unusual luminescence properties observed in copper-metallothionein from *Neurospora crassa* (K. Lerch, University of Zurich).

That metallothioneins transfer metal to

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*A post-FEBS 2-day international meeting on metallothionein (organized by J. Overnell and T.L. Coombs) was held on April 4 and 5 at the University of Aberdeen, Scotland.

apo-metalloproteins was supported by K. Lerch and by U. Weser and H. Hartmann (University of Tübingen). They showed reconstitution of different copper-dependent apoproteins with *Neurospora* and yeast copper-metallothionein respectively. However, no single function can be ascribed to metallothionein. M. Webb and K. Cain (MRC Toxicology Unit, Carshalton) demonstrated that in rats the protein can act as a mobile zinc and copper reserve in maintaining homeostasis or as a control agent for zinc and copper uptake in fetal and neonatal life stages. The detoxication role for controlling cadmium and mercury may well be a carry-over of this fetal mechanism into adult life.

Introducing the topic of metallothionein biosynthesis, R. Palmiter and D. Durham (University of Seattle) described experiments carried out on mouse metallothionein gene structure. Recombinant cDNA and genomic clones corresponding to mouse metallothionein-1 have been isolated and characterized. The DNA sequence indicates that this gene spans 1,100 base pairs with two intervening sequences (see Palmiter *Nature* 292; 267, 1981). The gene is regulated at the transcriptional level by heavy metals and less efficiently by glucocorticoid hormones both *in vivo* and in isolated cell lines.

Several cell lines that were selected for resistance to cadmium were shown to have amplified their metallothionein genes, allowing enhanced synthesis of this metal-binding protein. In an elegant study using DNA recombinant techniques, the regulatory portion of the mouse metallothionein gene was fused to the structural gene of thymidine kinase, rendering this enzyme inducible by Cd^{2+} . Furthermore, evidence was presented that the inducibility of the mouse metallothionein gene is controlled by DNA methylation.

The application of high-pressure liquid chromatography to the assessment of homogeneity, structural investigations and the isolation of fragments from enzymatic and chemical cleavage of metallothioneins was described by K.J. Wilson (University of Zurich). Other communications emphasized the importance of these metalloproteins in the homeostatic control of specific metals not only in mammalian species but also in yeasts, marine arthropods, crustaceans, eels and sea-birds. From the significant developments that were reported in all fields it seems that the elucidation of the mechanisms of synthesis, degradation, gene regulation and of the structural arrangements at the metal-binding sites of the metallothioneins is 'just around the corner'.

Treating urea cycle defects

from Vicente Rubio and Santiago Grisolia

THERE is much interest in the treatment of inborn errors of the urea cycle. As pointed out by Smith¹ in her excellent review, the prospects for patients with deficiencies of this cycle are far better now than in the recent past. Therefore, we regret her omission of *N*-acetylglutamate synthetase deficiency and of *N*-carbamoyl glutamate as a therapeutic agent for this condition.

N-acetylglutamate, synthesized by *N*-acetylglutamate synthetase, is the physiological activator of mitochondrial carbamoyl phosphate synthetase. Deficiency of *N*-acetylglutamate synthetase should induce hyperammonaemia. The deficiency cannot be treated by administration of *N*-acetylglutamate because this compound is deacetylated by cytosolic deacylases and it does not permeate the mitochondrial membrane. However, carbamoyl phosphate synthetase is also activated by analogues of *N*-acetylglutamate, such as *N*-carbamoyl glutamate², which is resistant to deacylases and which, when injected into rats, can be found in the mitochondrial matrix of liver cells³.

Therefore, it is not surprising that

Bachmann *et al.*⁴, who recently described the first case of hyperammonaemia due to *N*-acetylglutamate synthetase deficiency, found that *N*-carbamoyl glutamate is indeed extremely effective in the treatment of this condition.

As indicated by Smith, increases in the levels of *N*-acetylglutamate leading to greater activation of carbamoyl phosphate synthetase may improve control of ammonia levels in ornithine carbamoyl transferase, arginosuccinate synthetase and arginosuccinate lyase deficiencies and in partial defects of carbamoyl phosphate synthetase. Thus, although *N*-carbamoyl glutamate should be regarded as the specific treatment for *N*-acetylglutamate synthetase deficiency, its efficacy in these conditions should also be tested. *N*-carbamoyl glutamate may also be useful for patients with propionic or methylmalonic acidurias, for which decreased levels of *N*-acetylglutamate are postulated as a cause for the hyperammonaemia associated with these syndromes.

1. Smith, I. *Nature, News and Views* 291, 378 (1981).
2. Grisolia, S. & Cohen, P.P. *J. Biol. Chem.* 198, 561 (1952).
3. Rubio, V. & Grisolia, S. *Enzyme* (in the press).
4. Bachmann, C. *et al.* *New Engl. J. Med.* 304, 543 (1980).

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100 years ago

THE COMET OF 25 JUNE

THE appearance of a large comet has afforded an opportunity of adding to our knowledge of these bodies by applying to it a new means of research. Owing to the recent progress in photography it was to be hoped that photographs of the comet and even of its spectrum might be obtained and peculiarities invisible to the eye detected.

It was obvious that if the comet could be photographed by less than an hour's exposure there would be a chance of obtaining a photograph of the spectrum of the coma, especially as it was probable that its ultra-violet region consisted of but few lines. In examining my photographs of the spectrum of the voltaic arc, a strong band or group of lines was found above H, and on the hypothesis that the incandescent vapour of a carbon compound exists in comets, this band might be photographed in their spectrum.

Accordingly at the first attempt a photograph of the nucleus and part of the envelopes was obtained in seventeen minutes, on the night of June 24, through breaks in the clouds. On succeeding occasions, when an exposure of 162 minutes was given, the tail impressed itself to an extent of nearly ten degrees in length.

I next tried by interposing a direct-vision prism between the sensitive plate and the object-glass to secure a photograph which would show the continuous spectrum of the nucleus and the banded spectrum of the coma. After an exposure of eighty-three minutes a strong picture of the spectrum of the nucleus, coma, and part of the tail was obtained, but the banded spectrum was overpowered by the continuous spectrum.

I then applied the two-prism spectroscopy used for stellar spectrum photography, anticipating that, although the diminution of light would be serious after passing through the slit, two prisms, and two object-glasses, yet the advantage of being able to have a juxtaposed comparison-spectrum would make the attempt desirable, and, moreover, the continuous spectrum being more weakened than the banded by the increased dispersion, the latter would become more distinct. Three photographs of the comet's spectrum have been taken with this arrangement with exposures of 180 minutes, 196 minutes, and 228 minutes, and with a comparison spectrum on each. The continuous spectrum of the nucleus was plainly seen while the photography was in progress. For the present it suffices to say that the most striking feature is a heavy band above H which is divisible into lines, and in addition two faint bands, one between G and h, and another between h and H. I was very careful to stop the exposures before dawn, fearing that the spectrum of daylight might become superposed on the cometary spectrum.

It would seem that these photographs strengthen the hypothesis of the presence of carbon in comets, but a series of comparisons will be necessary, and it is not improbable that a part of the spectrum may be due to other elements.
HENRY DRAPER
271, Madison Avenue, New York
From *Nature* 24, 4 August, 308, 1881.

REVIEW ARTICLE

A one-receptor view of T-cell behaviour

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The discovery of T cells and their behaviour has forced a re-evaluation of the immunological relationship between self and not-self. T cells seem to respond against foreign antigens only when the latter are in some form of association with self molecules encoded by the major histocompatibility complex. This has raised the question of whether T-cell recognition may depend on two separate receptors. I present here the case for a model of T-cell behaviour based on a single receptor.

"I shall always regard the differentiation between self and not-self as crucial to all immunological theory."—F. M. Burnet.

Consider an immune response to a virus infection. B cells respond by producing antibodies which bind to virus particles and neutralize them. T cells, however, do not deal directly with the virus itself but instead interact with other cells. For example, T helper cells collaborate with B cells in the production of antibody while T killer cells prevent viral replication by lysing virus-infected cells.

These differences in T- and B-cell function necessitate different modes of antigen recognition. Whereas the receptors on B cells (antibodies) should be able to bind free virus particles, the receptors on T cells should not. A T cell which, like antibody, chases down the bloodstream after virus particles is redundant, and it is not surprising that T cells do not bind viruses or other antigens alone. Instead they recognize two entities: the foreign antigen in question (which makes them antigen specific) and a special cell-surface marker (which targets them onto cells).

The cell-surface markers that serve as T-cell guidance molecules¹ constitute a set of highly polymorphic membrane glycoproteins encoded by the major histocompatibility complex (MHC). Some (K and D) are found on almost all cells of the body and are specifically recognized by T killer cells, while others (the I antigens) are found mainly on cells of the immune system and guide T helper cells.

The recognition of MHC products by T cells is extremely precise. They distinguish between the products of self and foreign MHC alleles and their response to foreign molecules has long been the major barrier to successful tissue transplantation. They also recognize allelic differences when using their own MHC molecules as guidance proteins. For example, a virus-infected animal of MHC type 'A' contains activated T cells capable of recognizing and destroying only virus-infected cells which express the A allele. This is known as 'MHC restriction' of T-cell recognition.

A key question raised by MHC restriction concerns the nature of the T-cell receptor. Do T cells bear two separate receptors each of which recognizes one of the two components (antigen and MHC) or do they bear one receptor which recognizes a complex of the two molecules? The two-receptor viewpoint, known as dual recognition, has the advantage of requiring no special assumptions about the two antigens. They can simply be molecules floating freely in cell membranes. However, it has the disadvantage of requiring many assumptions about the genetics, structure, development and affinity of T-cell receptors. Nevertheless most models of T-cell behaviour have been constructed on the basis of dual recognition²⁻¹⁰.

It is time to reconsider the alternative known as the 'altered self' or 'interaction antigen' model¹¹⁻¹⁴. Based on a single T-cell receptor, it depends on one long-standing but difficult assumption about interactions between molecules in membranes. My

argument here is that this assumption still serves surprisingly well to explain the most important aspects of T-cell behaviour.

The assumptions

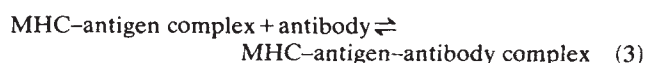
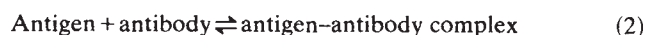
The assumption underlying the interaction antigen model is that T cells recognize neither antigen nor MHC molecules alone but rather molecular complexes composed of the two. This idea can be divided into two parts: (1) molecules in membranes can form associations (some transient, some more stable) which exhibit new antigenic determinants absent from the uncomplexed molecules; and (2) T cells are triggered to mature or differentiate only when they bind to MHC molecules on the surface of a specialized antigen-presenting cell.

Assumption (1)

Assumptions may be justified either because they make sense *a priori* or because of their ability to explain new data. I accept the idea of an interaction antigen purely on the basis of economy and predictive ability. First proposed¹⁰ in 1974, it has continued to explain many aspects of T-cell behaviour. A possible view of how such molecular interactions might occur was developed by Cohen and Eisen in 1977. They pointed out that, because cell membranes are essentially planar, the effective concentration of membrane molecules is exceedingly high. This high concentration generates a large number of molecular interactions, some of which will involve MHC molecules¹⁵.

A molecule floating freely in a membrane conforms to the ionic, hydrophobic and electrostatic environment. The same molecule, when complexed (however briefly) with another, will find itself in an altered environment and may thus take on a different conformation. In complexes of MHC and antigen, three kinds of new determinant may be formed: (1) changes in the conformation of the antigen, (2) changes in the conformation of the MHC protein, and (3) new surfaces involving both antigen and MHC.

If such complexes exist, why are they not often detected in co-capping studies? Most such complexes, based on high concentrations rather than innately high affinity, will not be very stable and may be detectable only in certain conditions. Consider three interacting molecules: an MHC protein, an antigen and antibody directed against the antigen. Three equations govern their interactions:



Depending on the antigenic determinant to which the antibody binds, the reaction depicted by equation (3) may predominate or not occur at all. The presence of an antibody directed against a determinant not available on the MHC-antigen complex will

reduce the amount of free antigen and drive all three equations to the left, diminishing the number of MHC-antigen complexes. An antibody with greater affinity for the complexed antigen than for the free form will stabilize the complex, driving equation (2) to the right and co-capping the MHC and the antigen¹⁶⁻¹⁹. These considerations should apply to T-cell receptors as well as to antibodies. In addition, the T-cell receptor, being membrane bound, may have a greater opportunity to achieve multipoint binding and thus further stabilize any molecular interaction to which it binds.

In principle, any molecule in the cell membrane should be able to interact with any other, yet the evidence is that T cells recognize only complexes that contain MHC molecules. This brings us to assumption (2).

Assumption (2)

Because recognition of MHC molecules is so important to T cells, it has been tempting to speculate that such recognition is genetically predetermined, that the germ-line genes code for a set of T-cell receptors directed specifically against MHC molecules. However attractive this assumption may seem, it cannot stand alone, and those who use it must explain (1) how T cells see non-MHC antigens, (2) how the gene pool manages to contain receptors able to bind new mutants in MHC molecules, and (3) how T cells choose from the gene pool those receptors which recognize the particular MHC allele(s) expressed by the body in which they live^{3-6,8,20}.

Because of these problems and, more importantly, because such a genetic bias is unnecessary, the interaction antigen model contains no such assumptions about the T-cell receptor gene pool and begins with a random repertoire (which may, in principle, contain receptors capable of binding any molecule alone or in combination with any other). How then are T cells restricted to recognition of antigen in the context of MHC molecules?

An early interaction antigen model¹¹ introduced the antigen-presenting cell as the determining factor in MHC restriction, proposing that T cells are selectively activated only when they bind to MHC molecules on the antigen-presenting cell surface (a concept generally accepted by all models of T-cell behaviour).

The general idea is that antigen-presenting cells participate actively in T-cell induction in two ways. First, they present antigens as well as MHC molecules to virgin T cells. Second, when a T cell binds to one of these MHC molecules, the antigen-presenting cell is induced to produce a differentiation signal. It is this differentiation signal which activates the T cell.

T cells should be able to bind in several ways to MHC molecules on the surface of antigen-presenting cells. Some will bind specifically to the MHC itself while others will bind to interaction determinants found on molecular complexes containing MHC. Like the antibodies discussed earlier, these latter may stabilize the complex, and the T cell will thus be treated like one which has bound free MHC. In general, any T cell which binds strongly to an MHC complex, no matter which part of the complex it binds, will be signalled to differentiate. Any T cell which binds any other complex or free molecule, failing to bind MHC, will not trigger the presenting cell and will not be activated. Thus, beginning with a random repertoire, one finishes with a functional set of T cells that are MHC restricted.

T-cell ontogeny and induction

Although all interaction antigen models are based on the same two assumptions, they differ in their development of specific points¹¹⁻¹⁴. In what follows I have tried to draw on those ideas which lead to the simplest overall view.

No assumptions were made about the germ-line genes coding for T-cell receptors. A simple idea would be that B-cell variable-region genes also code for T-cell receptors, but we can as easily start with a T-cell precursor population carrying any random set of specificities.

This repertoire of specificities must be modulated early in development to remove or inactivate T cells directed against self²¹⁻²³. Self tolerance is learned anew within each individual

and must involve at least two types of T cell: those specific for the self-MHC molecules alone and those specific for associations of other self molecules with self-MHC. It is unclear whether cells specific for isolated non-MHC molecules must be tolerized. Every potentially inducible cell must be tolerizable²⁴, but, as T cells specific for isolated non-MHC molecules fail to bind MHC, they are not inducible and should never be activated in the normal animal. However, were they to be activated by a cross-reactive complex of foreign antigen plus MHC, they could become autoreactive. One reason for supposing that such cells may not be tolerized is that it would require tolerance to have a different specificity from induction. Perhaps they form the basis of autoimmune disease. If so, one could predict that the destructive phase of some autoimmune diseases might not be MHC restricted.

Whether tolerance induction occurs in the thymus or in the periphery²⁵, and whether it occurs by mutation²⁰, deletion^{22,23} or suppression²⁶, parsimony suggests that the self antigens are presented by the same sort of antigen-presenting cell as those which present foreign antigens. The supposition then is that T-cell precursors are made tolerant when they bind to self-MHC molecules (or any complex containing self-MHC) on the surface of an antigen-presenting cell. This has the added attraction of allowing tolerance to occur to tissue antigens not normally intrinsic to cells of the immune system itself. Perhaps, as with B cells^{27,28}, an antigen recognition event may lead to tolerance in a young T-cell precursor and activation in a mature T cell.

Given a random repertoire depleted by self tolerance, several sorts of T cell will be available to bind to a foreign antigen X. Some will bind to X alone on the surface of the antigen-presenting cell but, failing to bind to MHC molecules, will not be activated. Others will bind in one of several ways to MHC-X complexes and will be activated. These T cells will retain their specificity and exercise their functions only when they encounter X in the same form as that with which they were activated, namely on the surface of a cell carrying the same MHC alleles.

MHC restriction

When Zinkernagel and Doherty first proposed 'altered self' recognition, the one thing known about MHC restriction was that T cells interacted only with syngeneic cells. The rules of T-cell behaviour are now better defined. How has the interaction antigen model held up?

Their original finding was that a mouse of MHC type A, immunized against an antigen X, contains activated T cells specific for targets carrying X+A and not targets carrying X+B^{10,29-35}. This simply reflects conservation of specificity—activated T cells respond best to the antigenic complexes with which they were primed. Because there are similarities between different MHC alleles as well as differences, X+A should have some determinants in common with X+B and a certain level of cross-reaction should exist. When T-cell responses are titrated far enough, such cross-reactions are usually seen³⁶⁻³⁹.

It has since been shown that MHC-restricted T cells do not bind free MHC or free antigen. Competition experiments show that neither free MHC nor free antigen will block T-cell activity^{32,40-43}. Furthermore, to be bound by T cells, MHC and antigen must not only be cell bound, they must be together in the same cell membrane^{29,44,45}. This is, of course, predicted if T cells recognize only interaction antigens, whereas dual recognition models must explain it *a posteriori*.

A further problem for at least some dual recognition models is posed by T-cell reactions against cells bearing foreign MHC. These models explain MHC restriction by supposing that T cells cannot function unless both of their receptors are engaged, one receptor being specific for antigen and the other for self MHC. However if a target carries only foreign MHC alleles, the anti-self receptor should be useless and the T cell helpless. To deal with this, some dual recognition models suggest that alloaggression is a 'special' case in which T cells need bind only one of their receptors. In contrast, the interaction model does not invoke such 'special cases'—T cells need only one receptor

whether recognizing a foreign-MHC antigen or some modification of a self-MHC product.

For similar reasons, it is easier to accommodate 'aberrant recognition'⁴⁶ in terms of the interaction antigen model. Since the original experiments on MHC restriction, it has become clear that T cells can recognize antigens presented with MHC molecules not found on their own tissues⁴⁷⁻⁵¹. These experiments are of course complicated by responses against the foreign MHC itself: an individual of MHC type A, immunized with antigen-presenting cells carrying MHC B and antigen X, makes such a strong response against B that it is virtually impossible to determine whether a response against antigen X also occurs. This complication can, however, be overcome by the removal of T cells reactive against B. In many experiments (but not all⁵²⁻⁵⁴), removal of such T cells reactive to B has revealed a population which can recognize X + B. In short, an animal that is itself MHC type A carries T cells capable of recognizing X + B.

This is predicted by the interaction antigen models. Because the specificity of the functional T-cell population is selected only at the time of antigen encounter, there is nothing to exclude the recognition of X + B. An antigen-presenting cell carrying B can activate T cells as efficiently as a presenting cell carrying A. Some dual recognition models can also incorporate this finding^{2,8}, but most ignore it.

It is not surprising that the interaction antigen idea deals well with MHC restriction as it was originally created for precisely that purpose. How does it fare with other aspects of T-cell behaviour?

Immune response genes

Immune response (Ir) genes code for MHC proteins and determine whether an animal can respond to a particular foreign antigen. For example, a mouse of MHC type A may respond to a particular antigen X, whereas a mouse of MHC type B does not⁵⁵⁻⁵⁸. The two assumptions underlying the interaction antigen view of MHC restriction allow at least three ways of explaining the Ir phenomena. (1) Some MHC alleles are able to form complexes with X while others, because of their structure, may create inappropriate environments for complexes to form. If no complex forms, no T cell can be activated (reviewed in ref. 59). (2) For any complex of X with MHC, there may be some determinants for which there is no T-cell receptor gene^{12,14,59}. (3) The induction of self tolerance creates gaps in the functional T-cell repertoire. The adult animal, having lost the ability to respond against its own tissues, cannot respond against antigens which cross-react with those tissues^{12,14}.

The first two explanations are straightforward. The third, while more complicated, can explain both MHC-linked and some non-MHC-linked immune response genes. It rests on the idea that a mouse of MHC type A is tolerant of many self components complexed with A. If an antigen X mimics self + A, the A-type mouse would be unable to respond to it. This does not mean that X itself must be identical to a 'self' component: it is the complex of X + A that is relevant. If X + A mimics any self + A complex, then an A mouse will not respond to X. However, an MHC B-type mouse may respond to X, implying that X + B carries some determinants not found on self + B.

How do these ideas stand up to our current understanding of Ir genes? An early model attributing nonresponsiveness to self tolerance predicted that responsiveness would be recessive⁶⁰ and was invalidated when responsiveness proved to be dominant. However, the discovery of MHC restriction allows a re-examination of this idea. If strain A is a nonresponder to X and strain B a responder, then explanation (3) above states that X + A mimics self + A while X + B carries determinants which do not cross-react with self + B. The F₁ progeny of an A × B mating will be tolerant of both self + A and self + B and will be unresponsive to X + A, but they will see the non-cross-reactive determinants on X + B and thus responsiveness will be dominant.

The crucial point is that, to T cells, X + A and X + B are entirely different antigens. Tolerance to one has no implications

for tolerance to the other and thus T cells of both responder and nonresponder genotypes should^{12,49,59} and do^{6,61-64} respond to X if it is presented with a responder-type MHC allele.

Some immune responses are governed by two MHC genes (α and β) which exhibit a peculiar complementation pattern in that some alleles complement while others do not⁶⁵⁻⁶⁹. (For example, if A, B and C are nonresponders, the (A × B)F₁ may respond while the (A × C) and (B × C) do not.) If α and β code for the two chains of the Ia guidance molecules^{70,71}, then the simplest explanation is that a particular pair of α and β chains forms an Ia molecule which can associate in a recognizable way with X, while another pair cannot. The (A × B)F₁ between two nonresponder strains will express the ineffective pair of Ia molecules of its parents ($\alpha^A\beta^A$ and $\alpha^B\beta^B$) and also two new types⁷² ($\alpha^A\beta^B$ and $\alpha^B\beta^A$). It is unpredictable whether a particular combination of α and β chains will form appropriate Ia molecules. However, some of the recombinant molecules may carry determinants which allow a response to X.

For some time all known MHC-linked Ir genes were located in the I region, provoking speculation that this might be the location of the T-cell receptor. However, Cunningham and Lafferty reasoned from the interaction antigen viewpoint that the known Ir genes mapped to the I region because the responses analysed had always been those of T helper cells, which use the I-region molecules as guidance molecules. They predicted that Ir genes for T killer responses would map to the K and D regions¹¹—a finding⁷³ now accepted as a matter of course.

Some T-cell responses are controlled by genes outside the MHC as well as by MHC-linked Ir genes⁷⁴⁻⁷⁸. Whereas most models ignore these non-MHC immune response genes, the tolerance model is predicated on their existence and predicts their genetic behaviour. If a mouse of MHC type A is unresponsive to X because X + A cross-reacts with self + A, then the lack of response is controlled by two genes: one coding for MHC 'A', the other for the 'self' cell-surface molecule. Why then do most Ir genes map only to the MHC? I presume that this is due to the lack of polymorphism in the genes coding for other cell components. If the non-MHC surface molecule (such as a hormone receptor) has no alleles, then every strain carrying MHC 'A' will be tolerant of the same self + A complex and response to X will seem to be exclusively controlled by the MHC genes. However, if alleles of the self molecule do exist, then some strains may be tolerant of an allelic form of self + A which does not mimic X + A. In this way two mice may differ in their responses to X even if both carry the same MHC product. The critical prediction from the tolerance model is that, unlike the MHC-linked Ir genes, the non-MHC immune response genes should lead to dominant unresponsiveness. The progeny of a nonresponder (self¹ + A) by responder (self² + A) mating will express and be tolerant of both self¹ and self² along with A. They should therefore be unresponsive to X.

Ir gene-controlled suppression: some mice appear to be unresponsive to an antigen because of suppression rather than deletion⁷⁹⁻⁸¹ and it has been suggested that this is inconsistent with cross-reactive self tolerance¹². However, I disagree. As self tolerance may be mediated or at least maintained by suppression^{82,83}, it is but a short step to suggest that nonresponsiveness due to cross-reactive self tolerance may also involve suppression.

Alloaggression

MHC molecules are themselves uniquely strong antigens⁸⁴⁻⁸⁸. Two decades ago it was suggested that this strength reflected a high frequency of reactive lymphocytes⁸⁶ and it is now known that as many as 10% of T cells can be specific for any foreign MHC molecule^{87,89-93}. Why is there a such high frequency of T cells specific for molecules which the individual meets only in artificial circumstances?

Although many explanations have been proposed^{2-6,8,11,20,94-99}, the interaction antigen model offers a new one¹³ which follows directly from the assumptions created for MHC restriction without any additions or special cases. The

argument is that if T cells from an MHC type-A mouse confront B-type antigen-presenting cells, they will recognize not only B but also all the complexes that B makes with the other molecules in the membrane. Thus the T cells are not responding only against the B antigens but against a very large number of antigenic complexes, and the frequency of reactive cells will be correspondingly high.

To analyse why this should occur let us return to self tolerance. A mouse is tolerant of its own MHC alleles and of all complexes they form with other self cell-surface molecules. It has no need and no opportunity to become tolerant of MHC type B either alone or complexed with other molecules. So, although mouse A is tolerant of its own components as seen with A, it will respond to these same components (as well as any new ones) associated with B. The result of immunizing with cells carrying foreign MHC molecules should thus be the massive and highly heterogeneous¹⁰⁰ response that is seen.

Does this interpretation account for other features of alloreactive responses? A single mutation at the MHC can elicit strong immune responses—this requires an explanation. It is known that in responses against conventional antigens, a single mutation at the MHC can profoundly change the T-cell response to the antigen associated with it¹⁰¹. For example, T cells directed against X+A may not recognize X+A^M (the mutant). By analogy, T cells tolerant of self+A may not be tolerant of self+A^M. The mutant MHC will interact with normal surface molecules to generate a set of interaction antigens different from those formed by the parental MHC type. The mutant cells will thus present a large number of antigenic determinants to be recognized by parental lymphocytes.

If the majority of alloreactive T cells do not recognize MHC products except as interaction antigens with other cell-surface molecules, one would expect that purified MHC molecules would not interfere with alloreactions at any concentration. Although a few alloreactive cells should be directed against the foreign MHC molecules themselves, the effect of blocking them would be too small to be detected reliably. It has, indeed, been shown that purified MHC molecules do not block alloreactions^{41,42}.

Muddying the waters

So far I have shown how two assumptions deal with the principal features of T-cell behaviour. There is, however, one set of experimental results which has been used as evidence against the interaction antigen viewpoint. These are the experiments showing thymic 'bending'.

Homozygous MHC type-A mice which are irradiated and then reconstituted with (A×B)F₁ bone marrow contain a peripheral set of F₁ T cells and also F₁ antigen-presenting cells that can present antigens in association with both A and B MHC molecules. The result of immunizing such a chimaera against antigen X should be the activation of two sets of T cells, one specific for X+A and the other for X+B. However, the experiments show that (A×B) T cells which have matured in an A animal respond preferentially to antigens associated with A, whereas maturation in a B animal confers a bias towards X+B^{6,62,102-105}. Further testing showed that the organ responsible for this bias was the thymus¹⁰⁵⁻¹⁰⁸. One interpretation has been that T-cell precursors are positively selected in the thymus^{3-9,20}. In an A thymus, T-cell precursors which bind to A would differentiate into functional T cells whereas those specific for B would not mature appropriately. Thus, even if X were later presented with both A and B, the response would be directed only against X+A.

Naturally such an interpretation is difficult for an interaction antigen model. There is no obvious way to select T cells for A+X or B+X in the absence of X. However, I disagree with the positive selection interpretation on two grounds. First, it depends on a special set of assumptions which are not required to explain other aspects of T-cell behaviour. Second, the 'aberrant recognition' experiments (see above) show that normal T

cells can recognize antigens presented with non-self MHC alleles. In fact, in one comparative study, T cells from (A×B)→A chimaeras did not respond against virus+B, while normal A-type T cells, from which cells reactive to B had been removed, made good responses to virus+B⁵¹. Both sets of T cells had matured in an A thymus, yet one was able to recognize X+B whereas the other was not.

So what is going on in the chimaeras? Obviously the thymus is having some effect on the T-cell repertoire long before encounter with antigen, but need it be a positive selection? Could it be the reverse?

Unlike the normal A mouse, an (A×B)→A chimaera must be tolerant of B, yet, early on, its thymus contains only A-type antigen-presenting cells¹⁰⁹. How then does tolerance to B occur? There are broadly two ways in which tolerance may be induced. Natural self tolerance may occur either by deletion of self-reactive cells and/or by the induction of suppressor cells that function to suppress anti-self responses. A possible clue to the mechanism of tolerance in the radiation chimaeras comes from another type of (A×B)→A chimaera: the classic neonatally tolerant mouse. Strain A mice which have been injected at birth with (A×B) cells are chimaeras^{110,111} tolerant of B¹¹² and often exhibit immune responses 'bent' towards A¹¹³⁻¹¹⁵. Tolerance to B in these chimaeras can involve suppressor cells¹¹⁶⁻¹¹⁹. Are the radiation chimaeras similarly suppressed¹²⁰? If this is the case, the suppression may extend to B+X (see refs 121, 122) while tolerance to A, occurring by the normal thymic route, allows a response to A+X.

Until these issues are resolved it does not seem appropriate to encumber the interaction antigen model with a new set of assumptions merely to explain a thymic positive selection which may not exist. The model predicts that the functional T-cell repertoire is the result of selection at the time of antigen encounter and I leave it at that.

Antigen encounters can be separated roughly into two sets: encounter with self antigens, which affects the repertoire by removing those specificities directed against self, and encounter with foreign antigen which increases the proportional representation of T cells reactive to the complex of that foreign antigen with MHC. If, unexpectedly, the thymic bias does turn out to reflect a positive selection for self MHC, then it will be time to act in accordance with current immunological practice—to add another assumption.

Recapitulation

The two basic assumptions are: (1) molecules in membranes interact to form new antigenic determinants, and (2) T cells are triggered when they bind to MHC molecules on an antigen-presenting cell (either for tolerance or reactivity).

T-cell development: (1) Pre-T cells enter the thymus and express receptors from a pool which may be as random as that of B cells (perhaps the same pool). (2) T cells capable of binding to MHC molecules on antigen-presenting cells in the thymus are inactivated, including T cells which recognize self non-MHC molecules in association with self MHC. All other T cells mature and peripheralize. (3) At the time of antigen encounter, T cells which bind to antigen in association with MHC molecules on the presenting cell are activated.

Ir genes: Three types of defect control immune responses: (1) the antigen cannot associate with the MHC molecule; (2) the T-cell receptor gene pool lacks a receptor for a particular interaction determinant; and (3) the complex of antigen+MHC mimics self.

Alloaggression: The high frequency of alloreactive cells exists because normal non-MHC membrane molecules associate with the MHC on foreign antigen-presenting cells to create a very large and heterogeneous set of antigens that in turn is recognized by a large and heterogeneous set of T cells.

Conjectures

A model is only as good as its predictions. The following predictions are not made by dual recognition models and are

therefore distinguishing characteristics of the single receptor/interaction antigen viewpoint.

First, it follows from assumption (1) that MHC-restricted antibodies should exist. If interaction antigens exist and can be recognized by T cells, they should be seen by B-cell receptors as well. MHC-restricted antibodies should be a small fraction of the total antibody made against any antigen and will often be missed; however, possible candidates do exist^{123,124}. It is the dual specificity of T cells which led to the suggestion that they express two receptors. If single antibodies can be MHC restricted, there should be no need to postulate two receptors for T cells.

Second, some T cells specific for MHC A associated with X should bind B + Y or even Y alone. There is no reason to expect that a new antigenic determinant will be unique to one particular complex. It has already been shown that some T cells specific for self MHC + X cross-react on allogeneic MHC, that is, $A + X = B^{125-128}$. A type of cross-reaction which would not be predicted by dual recognition models is $A + X = B + Y$ (or $A + X = Y$).

Third, some non-MHC-linked Ir genes⁷⁴⁻⁷⁸ should show dominant unresponsiveness (see the explanation of tolerance).

Fourth, a mouse of MHC type A should be able to respond to both self and non-self antigens complexed with MHC type B (this follows from the explanation for alloaggression).

Refutations

If any of the following are true, then the model presented here is wrong and cannot be saved with additional assumptions or any special pleading. (1) If T cells which are MHC restricted at one stage (such as effector function) are found to be unrestricted at an earlier stage (such as activation or tolerance). (2) If a somatic cell hybrid of two T cells of different restriction specificities has the mixed specificity, that is, if one parent sees X + A and the other sees Y + B and the hybrid sees in addition X + B and Y + A.

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Note added in proof: Very recently refutation (2) was tested¹²⁹. Not a single clone was found that had mixed specificity.

- Mitchison, N. A. in *Strategies of Immune Regulation* (ed. Sercarz, E.) (Academic, New York, 1980).
- Janeway, C. A. Jr, Wigzell, H. & Binz, H. *Scand. J. Immun.* **5**, 993 (1976).
- Langman, R. E. in *Rev. Physiol. Biochem. Pharmac.* **81**, 1 (1978).
- Cohn, M. & Epstein, R. E. *Cell. Immun.* **39**, 125 (1978).
- Blanden, R. V. & Ada, G. L. *Scand. J. Immun.* **7**, 181 (1978).
- Von Boehmer, H., Haas, W. & Jerne, N. K. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2439 (1978).
- Droege, W. *Immunobiology* **156**, 2 (1979).
- Williamson, A. R. *Nature* **283**, 527 (1980).
- Seshi, B. *Curr. Sci.* **48**, 919 (1979).
- Zinkernagel, R. M. & Doherty, P. C. *Nature* **251**, 547 (1974).
- Cunningham, A. J. & Lafferty, K. J. *Scand. J. Immun.* **6**, 1 (1977).
- Schwartz, R. H. *Scand. J. Immun.* **7**, 3 (1978).
- Matzinger, P. & Bevan, M. J. *Cell. Immun.* **29**, 1 (1977).
- Matzinger, P. thesis, Univ. California, San Diego (1980).
- Cohen, R. J. & Eisen, H. N. *Cell. Immun.* **32**, 1 (1977).
- Schrader, J. W., Cunningham, B. A. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5066 (1975).
- Bourguignon, L. Y., Hyman, R., Trowbridge, I. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2406 (1978).
- Zarling, D. A., Keshet, I., Watson, A. & Bach, F. H. *Scand. J. Immun.* **8**, 497 (1978).
- Gomard, E. *et al. Eur. J. Immun.* **8**, 228 (1978).
- Jerne, N. K. *Eur. J. Immun.* **1**, 1 (1971).
- Owen, R. D. *Science* **102**, 400 (1945).
- Billingham, R. E., Brent, L. & Medawar, P. B. *Phil. Trans. R. Soc.* **239**, 357 (1956).
- Burnet, F. M. *The Clonal Selection Theory of Acquired Immunity* (Cambridge University Press, 1959).
- Bretscher, P. & Kohn, M. *Science* **169**, 1042 (1970).
- Doherty, P. C. & Bennink, J. R. *Scand. J. Immun.* **12**, 271 (1980).
- Gershon, R. K. & Kondo, K. *Immunology* **21**, 903 (1971).
- Nossal, G. J. V. & Pike, B. L. *J. exp. Med.* **141**, 904 (1975).
- Lederberg, J. *Science* **129**, 1649 (1959).
- Bevan, M. J. *J. exp. Med.* **142**, 1349 (1975).
- Kindred, B. & Shreffler, D. C. *J. Immun.* **109**, 940 (1972).
- Katz, D. H., Hamaoka, T. & Benacerraf, B. *J. exp. Med.* **137**, 1405 (1973).
- Shearer, G. M. *Eur. J. Immun.* **4**, 527 (1974).
- Gordon, R. D., Mathieson, B. J., Samelson, L. E., Boyse, E. A. & Simpson, E. J. *exp. Med.* **144**, 810 (1976).
- Miller, J. F. A. P., Vadas, M. A., Whitelaw, A. & Gamble, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2486 (1976).
- Transplant. Rev.* **29** (1976).
- Peavey, D. L. & Pierce, C. W. *J. Immun.* **115**, 1515 (1975).
- Lindahl, K. F. & Wilson, D. B. *J. exp. Med.* **145**, 508 (1977).
- Teh, H. S., Phillips, R. A. & Miller, R. G. *J. Immun.* **120**, 425 (1978).
- Teh, H. S., Phillips, R. A. & Miller, R. G. *J. Immun.* **121**, 1711 (1978).
- Plata, F. & Levy, J. P. *Nature* **249**, 271 (1974).
- Todd, R. F. III, Stulting, R. D. & Amos, D. B. *Cell. Immun.* **18**, 304 (1975).
- Stulting, R. D., Todd, R. F. III & Amos, D. B. *Cell. Immun.* **20**, 54 (1975).
- Basten, A., Miller, J. F. A. P. & Abraham, R. J. *exp. Med.* **141**, 547 (1975).
- Swierkosz, J. E., Rock, K., Marrack, P. & Kappler, J. W. *J. exp. Med.* **147**, 554 (1977).
- Watt, T. S. & Gooding, L. R. *Nature* **283**, 74 (1980).
- Doherty, P. C. & Bennink, J. R. *J. exp. Med.* **149**, 150 (1979).
- Heber-Katz, E. & Wilson, D. B. *J. exp. Med.* **142**, 928 (1975).
- Pierce, C. W., Kapp, J. A. & Benacerraf, B. *J. exp. Med.* **144**, 371 (1976).
- Thomas, D. W. & Shevach, E. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2104 (1977).
- Wilson, D. B., Lindahl, K. F., Wilson, D. H. & Sprent, J. *J. exp. Med.* **146**, 361 (1977).
- Doherty, P. C. & Bennink, J. R. *J. exp. Med.* **150**, 1187 (1979).
- Sprent, J. & von Boehmer, H. *J. exp. Med.* **144**, 617 (1976).
- Shearer, G. M. & Schmitt-Verhulst, A. *Adv. Immun.* **25**, 55 (1977).
- Sprent, J. *Immun. Rev.* **42**, 108 (1978).
- Benacerraf, B. & McDevitt, H. O. *Science* **175**, 273 (1972).
- Lilly, F., Graham, H. & Coley, R. *Transplant. Proc.* **5**, 193 (1973).
- Davis, S., Shearer, G. M., Mozes, E. & Sela, M. *J. Immun.* **115**, 1530 (1975).
- Immun. Rev.* **38** (1978).
- Benacerraf, B. & Germain, R. *Immun. Rev.* **38**, 70 (1978).
- McDevitt, H. O. & Benacerraf, B. *Adv. Immun.* **11**, 31 (1969).
- Chesebro, B. W. *et al. Eur. J. Immun.* **2**, 243 (1972).
- Kappler, J. W. & Marrack, P. *J. exp. Med.* **148**, 1510 (1978).
- Longo, D. L. & Schwartz, R. H. *J. exp. Med.* **151**, 1452 (1980).
- Hodes, R. J., Hathcock, K. S. & Singer, A. J. *Immun.* **123**, 2823 (1979).
- Dorf, M. E. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3671 (1975).
- Günther, R. & Rüde, E. *J. Immun.* **115**, 1387 (1975).
- Melchers, I. & Rajewsky, K. *Eur. J. Immun.* **5**, 753 (1975).
- Dorf, M. E., Twigg, M. B. & Benacerraf, B. *Eur. J. Immun.* **6**, 552 (1976).
- Dorf, M. E. & Stimpfling, J. H. *J. exp. Med.* **146**, 571 (1977).
- Cook, R. G., Vitetta, E. S., Uhr, J. W. & Capra, J. D. *J. exp. Med.* **149**, 981 (1979).
- Silver, J. J. *Immun.* **123**, 1423 (1979).
- Fathman, C. G. & Hengartner, H. *Nature* **272**, 617 (1978).
- Simpson, E. & Gordon, R. D. *Immun. Rev.* **35**, 59 (1977).
- McDevitt, H. O. & Chinitz, A. *Science* **163**, 1207 (1969).
- Gasser, D. L. *J. Immun.* **105**, 908 (1970).
- Lilly, F., Jacoby, J. S. & Coley, R. C. in *Immunogenetics of the H-2 System* (eds Lengerová, & Vojtisková, M.) 197-199 (Karger, Basel, 1971).
- Gasser, D. L. & Silvers, W. K. *Adv. Immun.* **18**, 1 (1974).
- Keck, K. & Momayez, M. J. *Immun.* **121**, 1612 (1978).
- Kapp, J. A., Pierce, C. W., Schlossman, S. & Benacerraf, B. *J. exp. Med.* **140**, 648 (1974).
- Debré, P., Waltenbaugh, C., Dorf, M. E. & Benacerraf, B. *J. exp. Med.* **144**, 272 (1976).
- Benacerraf, B. & Dorf, M. E. *Cold Spring Harb. Symp. quant. Biol.* **41**, 465 (1976).
- Transplant. Rev.* **26** (1975).
- Immun. Rev.* **46** (1979).
- Gorer, P. A. *J. Path. Bact.* **47**, 231 (1938).
- Cunee, S., Smith, P., Barth, R. & Snell, G. D. *Ann. Surg.* **144**, 198 (1956).
- Simonsen, M. *Prog. Allergy* **6**, 349 (1962).
- Dutton, R. W. *J. exp. Med.* **123**, 665 (1966).
- Cantrell, J. L. & Hildemann, W. H. *Transplantation* **14**, 761 (1972).
- Nisbet, N. W., Simonsen, M. & Zaleski, M. J. *exp. Med.* **129**, 459 (1969).
- Ford, W. L., Simmonds, S. J. & Atkins, R. C. *J. exp. Med.* **141**, 681 (1975).
- Bevan, M. J., Langman, R. E. & Cohn, M. *Eur. J. Immun.* **6**, 150 (1976).
- Lindahl, K. F. & Wilson, D. B. *J. exp. Med.* **145**, 508 (1977).
- Swain, S. L., Panfili, P. R., Dutton, R. W. & Lefkowitz, I. J. *Immun.* **123**, 1062 (1979).
- Burnet, F. M. *Nature* **226**, 123 (1970).
- Thomas, L. in *Cellular and Humoral Aspects of the Hypersensitive States* (ed. Lawrance, S.) 529 (Cassell, London, 1959).
- Simonsen, M. *Cold Spring Harb. Symp. quant. Biol.* **32**, 517 (1967).
- Warner, N. L. *Cold Spring Harb. Symp. quant. Biol.* **32**, 523 (1967) (Discussion).
- Burakoff, S. J. *et al. J. exp. Med.* **148**, 1414 (1978).
- Cunningham, A. J. *Cell. Immun.* **19**, 368 (1975).
- Sherman, L. A. *J. exp. Med.* **151**, 1386 (1980).
- Klein, J. *Adv. Immun.* **26**, 55 (1978).
- Bevan, M. J. *Nature* **269**, 417 (1977).
- Sprent, J. *J. exp. Med.* **147**, 1838 (1978).
- Zinkernagel, R. M. *et al. J. exp. Med.* **147**, 882 (1978).
- Waldmann, H., Pope, H., Bettles, C. & Davies, A. J. S. *Nature* **277**, 137 (1979).
- Zinkernagel, R. M. *et al. J. exp. Med.* **147**, 897 (1978).
- Fink, P. J. & Bevan, M. J. *J. exp. Med.* **148**, 766 (1978).
- Miller, J. F. A. P., Gamble, J., Mottram, P. & Smith, F. I. *Scand. J. Immun.* **9**, 29 (1979).
- Longo, D. L. & Schwartz, R. H. *Nature* **287**, 44 (1980).
- Lubiaroff, D. M. & Silvers, W. K. *J. Immun.* **111**, 65 (1973).
- Nakić, B., Mikuška, J., Kaštelan, A., Springer, O. & Silobrić, V. *Immunology* **18**, 119 (1970).
- Brent, L., Brooks, C. G., Medawar, P. B. & Simpson, E. *Br. med. Bull.* **32**, 101 (1976).
- Kindred, B. *Cell. Immun.* **20**, 241 (1975).
- Zinkernagel, R. M., Callahan, G. N., Streilein, J. W. & Klein, J. *Nature* **266**, 837 (1977).
- Waldmann, H., Pope, H., Brent, L. & Bighouse, K. *Nature* **274**, 166 (1978).
- Roser, B. J. & Dorsch, S. *Nature* **258**, 233 (1975).
- Gorczynski, R. M. & MacRae, S. J. *Immun.* **122**, 747 (1979).
- Holán, V., Chutná, J. & Hášek, M. *Nature* **274**, 895 (1978).
- Reiger, M. & Hilgert, I. J. *Immunogenetics* **4**, 61 (1977).
- Smith, F. I. & Miller, J. F. A. P. *J. exp. Med.* **151**, 246 (1980).
- Yowell, R. L., Araneo, B. A., Miller, A. & Sercarz, E. E. *Nature* **279**, 70 (1979).
- Schwartz, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2862 (1976).
- Ivanyi, P., Melief, C. J. M., van Mourik, P., Vluga, A. & de Greeve, P. *Nature* **282**, 843 (1979).
- van Leeuwen, A., Goulmy, E. & van Rood, J. J. *J. exp. Med.* **150**, 1075 (1979).
- Bevan, M. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2094 (1977).
- Lemonnier, F., Burakoff, S. J., Germain, R. N. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1229 (1977).
- Finberg, R., Burakoff, S. J., Cantor, H. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5145 (1978).
- Von Boehmer, H. *et al. Eur. J. Immun.* **9**, 592 (1979).
- Kappler, J. W., Skidmore, B., White, J. & Marrack, P. *J. exp. Med.* **153**, 1198 (1981).

ARTICLES

Effect of ions on the light-sensitive current in retinal rods

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The effect of ions on the light-sensitive current of retinal rods was studied by sucking the inner segment into a tightly fitting capillary with the outer segment projecting into a flowing solution. This new method showed that the light-sensitive pathway, in which Na^+ is the normal carrier of current, has an ionic selectivity different from that of other known sodium channels. External calcium has a striking effect on the current, which increased about 20-fold when all calcium was removed. Reducing the sodium concentration gradient greatly prolonged the response to a flash of light, as would be expected if internal calcium blocks sodium channels and if light releases calcium which is subsequently extruded by a sodium-calcium exchange mechanism.

THE experiments described here were carried out with a new method which gives quantitative information about the effects of ions on the current generated by an isolated photoreceptor. The method is an extension of that developed by Baylor, Lamb and Yau^{1,2} and in its usual form depends on sucking the inner segment of an isolated rod into a recording capillary while leaving the outer segment projecting into a flowing solution whose composition can be changed rapidly. This arrangement (Fig. 1a) allows one to examine the effects of substances on the light-sensitive pathways in the outer segment; similar experiments on the inner segment may be carried out by inverting the cell as in Fig. 1b. Light responses obtained with the two arrangements were of similar shape and size and of the same polarity with respect to the rod.

A piece of retina from a dark-adapted toad (*Bufo marinus*) was chopped into small pieces in Ringer's solution under IR light, yielding a preparation that consists of many detached outer segments, which did not give light responses, and a few fairly complete cells, that is, outer segments still attached to inner segments containing a nucleus but without synaptic endings. When the inner segment of such a cell is sucked into a closely fitting pipette containing Ringer's solution it gives currents of the usual form and magnitude for several hours.

In addition to changing the light-sensitive current, test solutions may set up a junctional current that arises from the small difference between the junctional potentials at the two boundaries of the test solution. Unlike the light-sensitive current, the junctional current did not change sign when the rod was inverted, nor was it much altered by replacing a complete rod by an inert outer segment. When the results were corrected for this junctional current, it was found that changing external ions altered the dark current but usually had little effect on the current in the presence of a bright light, which remained close to zero.

The records in Fig. 1 show that halving the sodium concentration round the outer segment reduced the light response to one-third but had no effect on the response when applied to the inner segment. This illustrates the difference between the two parts of the cell and shows that there was no appreciable leakage of ions through the tight seal at the mouth of the capillary.

Ionic selectivity of the light-sensitive channel

As expected from previous work³⁻⁹, replacement of sodium by choline in the solution bathing the outer segment abolished the light response in a rapid and reversible manner. Figure 2 shows the relationship between the maximum response and external sodium concentration with 1 mM Ca in the external solution. A similar curve is obtained with 0.1 mM Ca in the external solu-

tion, except that the currents are scaled up by a factor of 3-5 (see below).

We looked for an inverted response in the complete absence of external sodium but could detect no response of either polarity in sodium-free choline solutions containing the normal amount of external calcium (1 mM). This failure to see an inverted response may be due to (1) the recording limit of ~ 0.1 pA, (2) the probable hyperpolarization when the dark current is cut off in low external sodium, (3) the possible closure of sodium channels resulting from the rise in internal calcium which might follow removal of external sodium¹⁰. The responses seen in sodium-free solutions containing 0 Ca-EGTA are discussed below.

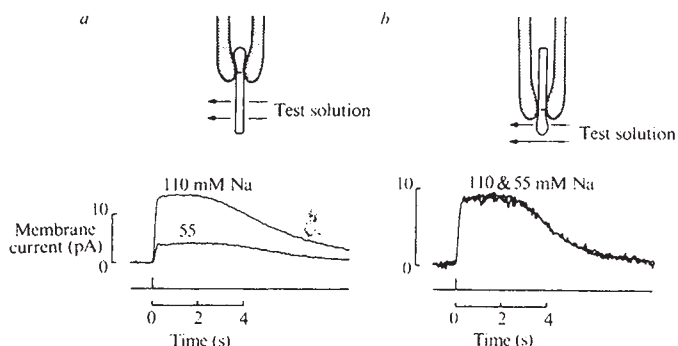


Fig. 1 a, Above, recording arrangement with the rod inner segment drawn into the Ringer-filled pipette and the outer segment exposed to test solutions. Membrane current was recorded with a current-to-voltage transducer connected to the pipette. Below, averaged responses to identical bright flashes in the presence of either 110 mM Na or 55 mM Na + 55 mM choline. In plotting the traces the baseline has been shifted so that the dark levels superimpose. b, Experimental conditions similar to a, except that the rod outer segment was inside the pipette and the inner segment exposed to test solutions. Outward membrane current across the outer segment is taken as positive (upwards) in both cases; responses are low-pass filtered at 30 Hz (6-pole); temperature 19 °C. Time separation between recordings in a and b (which were obtained on the same rod) was ~ 2 h, during which the maximum response of the cell had declined somewhat. Light monitor trace below the responses indicated timing of 20-ms flash ($\lambda = 500$ nm). The flashes delivered 37 photons μm^{-2} (unpolarized), corresponding to 740 photoisomerizations (Rh^*) per flash for an outer segment with an effective collecting area of about $20 \mu\text{m}^2$. The Ringer's solution in all experiments contained (mM): NaCl, 110; KCl, 2.5; MgCl_2 , 1.6; CaCl_2 , 1.0; HEPES (neutralized with tetramethylammonium hydroxide to pH 7.6), 10; glucose, 3. Test solutions were similar except for the specific substitutions stated. Calcium buffers were made with EGTA, HEDTA and Mg-EDTA to cover a range of 10^{-5} – 10^{-8} M Ca^{2+} using the stability constants in ref. 29. The seal resistance with the rod in position was 12 M Ω in a and 8 M Ω in b. In other experiments seal resistances varied between 5 and 15 M Ω .

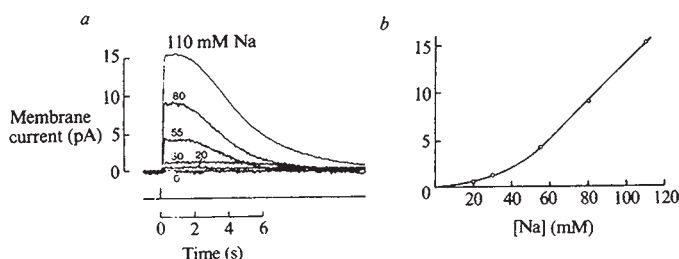


Fig. 2 Dependence of light-sensitive current on external sodium. *a*, Responses of an isolated rod to bright flashes (delivering $1,534 \text{ Rh}^*$ per flash) with different sodium concentrations bathing the outer segment. Temperature 19°C . Choline was used as substitute in the low-sodium solutions. The 110 mM Na solution (Ringer) was applied between different low-sodium solutions and the response in *a* represents the grand average of 34 responses all within the range 14–16 pA. The other responses are averages over 3–10 flash trials. Note the prolongation of the plateau at external sodium concentrations $< 55 \text{ mM}$. *b*, Plot of the response peaks in *a* versus external sodium concentration. 1 mM Ca^{2+} present throughout.

Lithium was able to substitute for sodium in carrying the inward dark current, but was less effective. Complete replacement of sodium with lithium reduced the current from 15.4 to 0.6 pA in a representative experiment. With a solution in which all but 20 mM of sodium had been replaced by choline the current was 0.56 pA. As 20 mM Na is almost as effective as 110 mM Li we conclude that the permeability ratio $P_{\text{Li}}/P_{\text{Na}}$ is about 1:5 in these conditions. A similar ratio was also obtained with sodium present throughout. In the presence of 55 mM Na, replacing 55 mM choline with lithium caused an increase in current from 4.0 to 6.3 pA. Earlier in the same experiment an increment in sodium concentration from 55 to 80 mM caused an increase in current from 4.2 to 9.0 pA. Assuming that the current increase was proportional to the sodium increment in this concentration range (see Fig. 2), we calculate that 13 mM Na is equivalent to 55 mM Li, and therefore that the permeability ratio is 1:4.2. The permeability ratio was also not significantly affected by reducing the external calcium to as low as $1 \mu\text{M}$ even though in low calcium the dark current is greatly increased.

The other alkali cations behaved quite differently. With zero external sodium, application of 110 mM potassium, rubidium or caesium produced no detectable current in the presence of 1 mM Ca , but at lower concentrations small currents were observed. In $100 \mu\text{M Ca}$, tentative values of the permeability ratios were $P_{\text{K}}/P_{\text{Rb}}/P_{\text{Cs}}/P_{\text{Na}} = 0.02:0.015:0.01:1.0$. In the presence of external sodium, however, potassium and to a lesser extent rubidium, but not caesium, actually reduced the existing dark current. In one experiment, increasing potassium from 2.5 to 20 mM, with sodium present as the main cation in both cases,

reduced the dark current to 36%. This effect cannot be attributed to potassium leaking into the pipette and depolarizing the inner segment because when the rod was inverted in the pipette and the same solutions applied to the inner segment, the current was reduced only to 75%. It therefore seems that external potassium and rubidium ions have some kind of blocking action on the inward sodium currents in the rod outer segment.

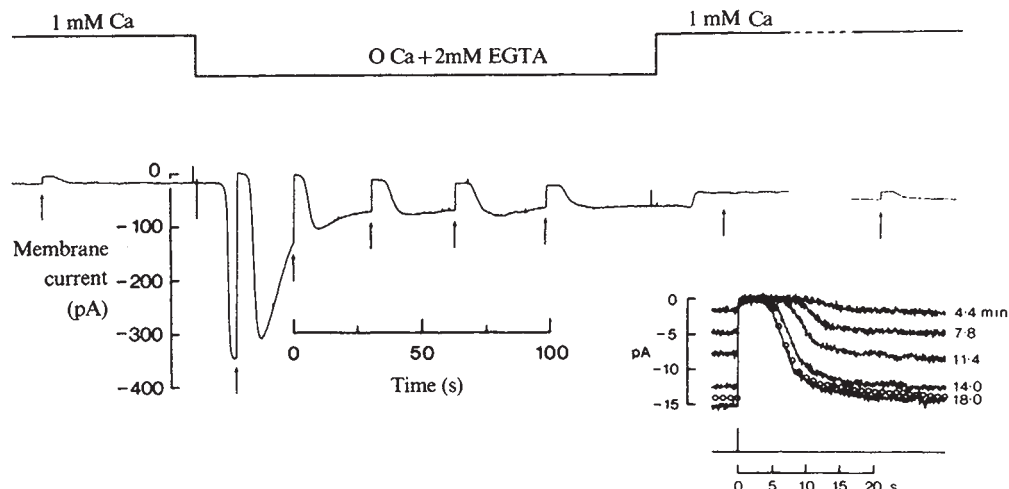
These results show that the sodium conductance in rods differs in its ionic selectivity from other known sodium conductances. In nerve, for example, lithium substitutes equally for sodium^{11–13} and external potassium has no direct blocking effect¹⁴. It remains for future work to show whether the light-sensitive conductance is a pore or a carrier system.

Effects of external calcium on dark current

In agreement with Yoshikami and Hagins¹⁵, altering the external calcium concentration caused large changes in dark current and light response. In Fig. 3, 0 Ca plus 2 mM EGTA increased the dark current and light response from 15 to 340 pA. This large dark current, which depended on external sodium, was not maintained probably because the cell gained sodium and lost potassium. On restoring Ringer's solution, the light response often disappeared completely for a few minutes and when it returned it was smaller and much longer than normal. However, a full sized response was usually restored within 10–20 min or sooner if the exposure to 0 Ca was shorter. Details of the changes in response during recovery are given in the inset of Fig. 3. Evidence that the lengthening of response and reduction in photocurrent after a period in low calcium were caused by entry of sodium is provided by the facts that they occurred only if sodium was present during the period in low calcium, and they disappeared if the rise in dark current was prevented by exposure to a bright light during the application of 0 Ca.

Raising external calcium from 1 to 5 mM decreased the light-sensitive current to about one-quarter and lowering it from 1 to 0.1 mM increased the current by a factor of 3–6. In one rather complete experiment, replacing 1 mM Ca with $10 \mu\text{M Ca}$ increased the current from 20 to 170 pA whereas 0 Ca-EGTA gave 320 pA, both measurements being repeated twice. The initial effect of altering calcium concentration was to scale the quantal response in proportion to the dark current. In the above experiment, tests with weak flashes showed that the peak current produced by absorption of a single quantum was 0.1 pA in 5 mM, 0.35 pA in 1 mM and 2.5 pA in $10 \mu\text{M Ca}$. The approximate proportionality between the quantal response and dark current indicates that the fraction of channels blocked by a single photon remains roughly constant in spite of the large increase which calcium reduction must produce either in the total number of sodium channels open in the dark or in the conductance of individual channels.

Fig. 3 Low-gain record showing the effect of removing external calcium on the photocurrent in a toad rod. Inward current is shown downwards from the level in bright flashes. Flashes each giving $5.2 \times 10^4 \text{ Rh}^*$ were delivered at times indicated by arrows. Mg was present throughout at 1.6 mM, K at 2.5 mM and Na at 110 mM. Inset, details of the same experiment showing recovery of dark current and light response (unaveraged) in Ringer's fluid. Numbers give the time after restoring Ringer; open circles show the response before applying 0 Ca. Inward current shown downward from the level after bright flash, which remained constant during the recovery period.



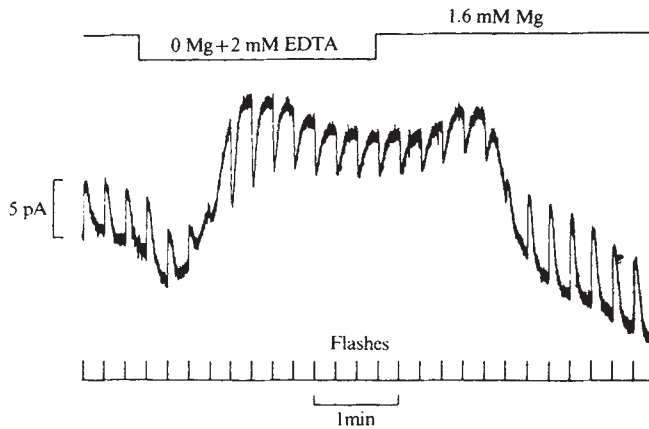


Fig. 4 Inversion of rod light response in 0 Mg and 0 Ca solution. Outward membrane current across the outer segment is upward. Solutions contained 110 mM choline chloride, 2.5 mM KCl, 0 Ca, 2 mM EGTA and 1.6 mM MgCl_2 or 0 Mg with 2 mM EDTA. The bright flashes, which elicited saturating responses, were estimated to give $6,500 \text{ Rh}^*$ in the outer segment. The solution change in this experiment was slower than in other experiments, with a time lag between change in tap position and solution change around the outer segment of about 0.5 min.

Experiments with a rapid flow system in which external calcium was changed from 1 to 5 mM and back showed that the restoration of dark current lagged behind the solution change at the pipette by ~ 1 s and that the restoration of dark current after reducing calcium occurred much faster than the return of current after a bright flash. The following values were obtained in two experiments with a particularly fast flow: time of solution change measured between 10 and 90% limits = 0.6 s; time for dark current to increase between the same limits after lowering $[\text{Ca}]_0$ from 5 to 1 mM = 1.6 s; time for dark current to increase between same limits after bright flash = 10 s. Another relevant comparison is that the maximum rate of increase of current on reducing calcium from 5 to 1 mM was 9 pA s^{-1} , whereas the corresponding value after a bright flash was 1.6 pA s^{-1} . In these experiments the speed of the solution change was obtained from the change in junctional current measured in the presence of a bright light.

The speed with which external calcium ions affect the sodium conductance is explained simply if they act directly on the outside of the membrane, but an internal action cannot be ruled out. If calcium ions act internally, as has sometimes been

proposed¹⁶, one must elaborate the calcium transmitter hypothesis to accommodate the observation that the recovery of dark current after a bright flash is so much slower than the recovery when external calcium ions are reduced. One modification is to assume that calcium release continues after the light flash is over. Another is to suppose that calcium shuts channels, but that a more complicated process is involved in opening them again.

Light responses in the absence of external sodium

Using voltage recording, E. R. Griff, L. H. Pinto, B. L. Bastian and G. L. Fain (personal communication) showed that in solutions containing calcium buffered to 10^{-8} M with EGTA, replacement of sodium by choline did not abolish the response but gave a residual response of normal polarity. We have observed the same phenomenon when recording current but only if the choline-EGTA solution contained magnesium. With no magnesium we obtained an inverted response, that is, an outward dark current that was suppressed by light (Fig. 4). These magnesium-sensitive currents appeared only if the calcium concentration was less than $\sim 1 \mu\text{M}$. Their appearance might be explained if the effects of calcium removal on cation conductance are brought about by unmasking negatively charged sites which attract sodium and magnesium ions into or near the light-sensitive channel. Thus, if calcium removal created a negative potential well large enough to increase sodium permeability by 20, it should on a simple basis increase magnesium permeability by 400. The effect of magnesium removal in inverting the EGTA response has recently been confirmed in similar conditions with intracellular voltage recording (V. Torre, E. Pasino, M. Capovilla and L. Cervetto, personal communication).

Effects of alterations of external and internal sodium

Alterations in external sodium have a striking effect on the time course of the light response. Reducing the sodium concentration from 110 to 20 mM prolonged the response to strong and weak flashes (Fig. 5). It also made the cell more sensitive in the sense that low sodium increased the fraction of the maximum current suppressed by each absorbed photon. The correlation between time to peak and amplitude illustrated by the scaled responses in Fig. 5c is reminiscent of the desensitizing effects of background

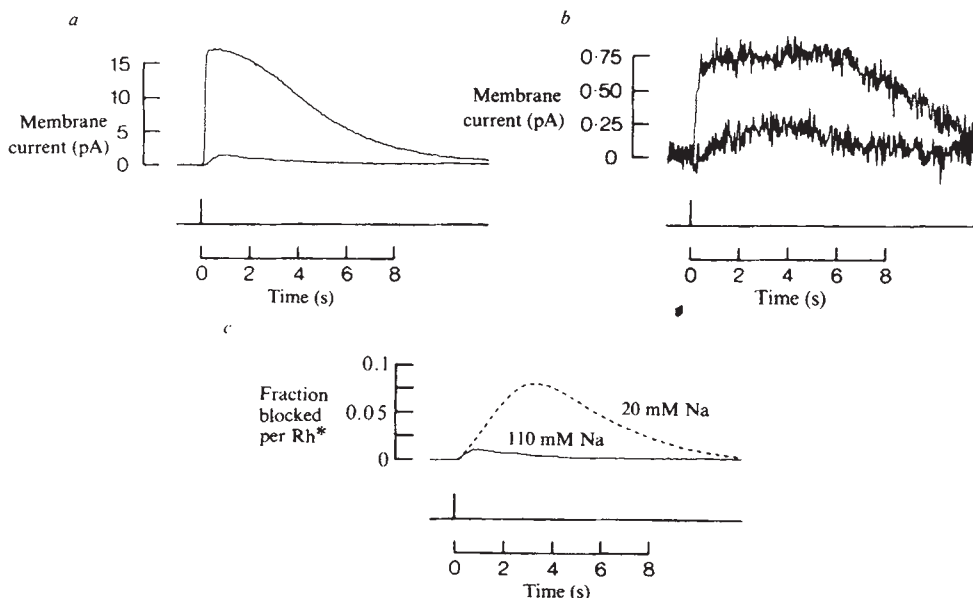


Fig. 5 Change in sensitivity and response time course in the presence of low external sodium. *a*, Averaged responses to bright and dim flashes in Ringer's solution (110 mM Na) before and after applying low sodium. Bright flash gave $1,660 \text{ Rh}^*$ and dim flash 8.1 Rh^* . *b*, Averaged responses to bright and dim flashes in 20 mM Na. Bright flash gave $1,660 \text{ Rh}^*$ and dim flash 4.14 Rh^* . Note the substantial prolongation of the responses. 1 mM Ca present throughout. *c*, Comparison of dim flash responses in 110 mM and 20 mM $[\text{Na}]_0$. Responses have been scaled to show the fraction of dark current blocked by one photoisomerization (Rh^*). Response in low $[\text{Na}]_0$ (dotted curve) smoothed by eye.

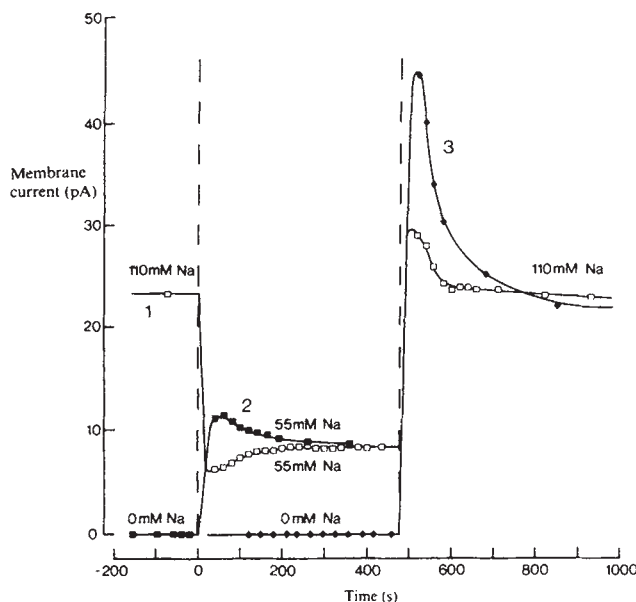


Fig. 6 Time course of change in response amplitude to bright flashes (1,660 Rh* per flash) with partial or complete replacement of external sodium by choline. 1 mM Ca present throughout. Note overshoot when sodium is increased and undershoot when it is decreased. Curve 1 ($\square$): $[\text{Na}]_o$ was 110 mM up to time 0, then 55 mM for 480 s and finally 110 mM again. Curve 2 ($\blacksquare$): $[\text{Na}]_o$ was 0 from -480 s to time 0 and then 55 mM for 480 s. Curve 3 ($\bullet$): $[\text{Na}]_o$ was 0 from time 0 to 480 s and then 110 mM. The three curves were obtained consecutively on the same rod.

lights¹⁷, with an increase in sodium gradient being roughly equivalent to a rise in background light intensity.

The effects of external sodium on response duration can be explained if a flash of light releases calcium ions which are then extruded to the external solution by sodium-calcium exchange¹⁸⁻²². A similar argument can account for the greatly prolonged responses seen in solutions in which external sodium is replaced by lithium.

Conditions which should increase internal sodium concentration, such as treatment with strophanthidin or previous exposure to low calcium (Fig. 3), also slowed the response to light flashes. This might be explained if internal sodium inhibited calcium transport across either disk or surface membrane.

The transient component in the response to changes in external sodium and calcium

A striking feature of the light-sensitive current is that after a change in external sodium or calcium the current does not approach its steady value monotonically but with an overshoot or undershoot of the kind shown in Fig. 6. An overshoot is seen when the dark current is raised by increasing external sodium or reducing external calcium and an undershoot when the dark current is decreased by ionic changes of the opposite kind. Figure 6 illustrates the effect of changing $[\text{Na}]_o$ at 1 mM Ca. The phenomenon is suggestive of a regulatory system in which sodium conductance varies inversely with internal sodium concentration. Such an effect might depend either on a direct inhibitory action of internal sodium or an indirect one, possibly mediated by the changes in internal calcium concentration that are likely to follow changes in internal sodium concentration²³.

For small perturbations from the normal condition in Ringer's solution, the half time with which the dark current returns is about 1 min, but shorter values and steeper-than-exponential return are seen for large overshoots (as in Fig. 6), and much longer half times and an S-shaped return after conditions likely to give a large increase in internal sodium, for example, after several minutes in 110 mM Na + 0 Ca.

Possible effect of reducing internal calcium on sensitivity to light

Exposure of >10 min to solutions such as 20 mM Na, 0 Ca, 0 Mg, 2 mM EGTA, sometimes reduces the sensitivity of the rod by several orders of magnitude, perhaps because the disks in the rod have lost most of their calcium²⁴. In these conditions the rod exhibits the phenomenon of 'superlinearity' in which doubling the intensity of a weak flash may increase the response by a factor of ≥ 10 . This behaviour might be explained by assuming that (1) several calcium ions are required to block each sodium channel, (2) with normal calcium one photon releases sufficient calcium ions to block many channels in a small region, and (3) after treatment with 0 Ca-EGTA the disks contain so little calcium that several photons must be absorbed simultaneously in a small region to release enough calcium to block any sodium channels at all.

Conclusions

These experiments with isolated rods support the widely held view that sodium ions normally carry the light-sensitive current into the outer segment of vertebrate photoreceptors. They also show that the current which flows in normal conditions is only a small fraction of that observed in low calcium. When taken with the rapid effect of external calcium on sodium conductance the result may imply that the sodium pathway is closed for about 95% of the time by combination with external calcium ions, and that light blocks the pathway completely by closing the inside for 100% of the time. Such a mechanism might allow a finer grading of response and less dark noise than if there were fewer pathways open for most of the time.

The present experiments do not prove that calcium is the internal blocking agent released by light, as first suggested by Hagins²⁵, nor do they provide any information about the role of cyclic GMP, another potential transmitter²⁶⁻²⁸. They do, however, provide some indirect evidence for the calcium hypothesis, particularly when taken in conjunction with recent work on the light-activated extrusion of calcium^{18,19}. Thus, the strong influence of the sodium concentration gradient on the time course of the light response fits rather well into a scheme in which some of the calcium ions released by light are transported to the outside by exchange with external sodium. The powerful effects of calcium ions in suppressing sodium current when applied externally also makes it easier to assume that they may have a somewhat similar effect on the inside of the membrane.

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1. Yau, K.-W., Lamb, T. D. & Baylor, D. A. *Nature* **269**, 78-80 (1977).
2. Baylor, D. A., Lamb, T. D. & Yau, K.-W. *J. Physiol., Lond.* **288**, 589-611 (1979).
3. Arden, G. B. & Ernst, W. *Nature* **223**, 528-531 (1969).
4. Sillman, A. J., Ito, H. & Tomita, T. *Vision Res.* **9**, 1443-1451 (1969).
5. Yoshikami, S. & Hagins, W. A. *Abstr. 14th Int. Meet. biophys. Soc.* WPM-13 (1970).
6. Korenbrot, J. I. & Cone, R. A. *J. gen. Physiol.* **60**, 20-45 (1972).
7. Cervetto, L. *Nature* **241**, 401-403 (1973).
8. Capovilla, M., Cervetto, L., Pasino, E. & Torre, V. *J. Physiol., Lond.* **317**, 223-242 (1981).
9. Brown, J. E. & Pinto, L. H. *J. Physiol., Lond.* **236**, 575-591 (1974).
10. Fain, G. L. & Lisman, J. E. *Prog. Biophys. molec. Biol.* **37**, 91-147 (1981).
11. Hodgkin, A. L. & Katz, B. *J. Physiol., Lond.* **108**, 37-77 (1949).
12. Chandler, W. K. & Meves, H. *J. Physiol., Lond.* **180**, 788-820 (1965).
13. Hille, B. *J. gen. Physiol.* **59**, 637-658 (1972).
14. Frankenhaeuser, B. *J. Physiol., Lond.* **160**, 40-45 (1962).
15. Yoshikami, S. & Hagins, W. A. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 245-255 (Springer, New York, 1973).
16. Hagins, W. A. & Yoshikami, S. *Ann. N.Y. Acad. Sci.* **307**, 545-561 (1978).
17. Baylor, D. A., Mathews, G. & Yau, K.-W. *J. Physiol., Lond.* **309**, 591-621 (1980).
18. Gold, G. H. & Korenbrot, J. I. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5557-5561 (1980).
19. Yoshikami, S., George, J. S. & Hagins, W. A. *Nature* **286**, 395-398 (1980).
20. Blaustein, M. P. & Hodgkin, A. L. *J. Physiol., Lond.* **200**, 497-527 (1969).
21. Baker, P. F. & McNaughton, P. A. *J. Physiol., Lond.* **259**, 103-144 (1976).
22. Daemen, F. J. M., Schneitkamp, P. P. M., Hendriks, Th. & Bonting, S. L. in *Vertebrate Photoreception* (eds Barlow, H. B. & Fatt, P.) 29-40 (Academic, London, 1977).
23. Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, R. A. *J. Physiol., Lond.* **200**, 431-458 (1969).
24. Lipton, S. A., Ostroy, S. E. & Dowling, J. E. *J. gen. Physiol.* **70**, 747-770 (1977).
25. Hagins, W. A. *Rev. Biophys. Bioengng* **1**, 131-158 (1972).
26. Hubbell, W. L. & Bownds, M. D. *A. Rev. Neurosci.* **2**, 17-34 (1979).
27. Lipton, S. A., Rasmussen, H. & Dowling, J. E. *J. gen. Physiol.* **70**, 771-791 (1977).
28. Miller, W. H. & Nicol, G. D. *Nature* **280**, 64-66 (1979).
29. Martell, A. E. & Smith, R. M. *Critical Stability Constants Vol. 1* (Plenum, New York, 1974).

The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes

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The Harvey and Kirsten strains of murine sarcoma virus encode enzymatically and serologically related p21 src proteins which are required for virally mediated cellular transformation. The genes in each virus encoding p21 show such extensive divergence from each other that cloned probes from these genes detect distinct sets of cellular genes in the DNA from several vertebrate species. These data suggest that cellular p21 src genes constitute a divergent family of vertebrate genes that can regulate the growth of cells.

THE Harvey and Kirsten strains of murine sarcoma virus (Ha-MuSV and Ki-MuSV) are replication-defective retroviruses that were isolated from independent mouse tumours induced by murine leukaemia viruses which had been passaged through rats^{1,2}. Ha-MuSV and Ki-MuSV are capable of both transforming fibroblasts in cell culture and inducing sarcomas and erythroleukaemias in susceptible mice³. The only known gene product of the viruses is a 21,000-molecular weight (MW) protein (p21) which is required for cellular transformation⁴ and is present at relatively high levels in virally transformed cells; a related but distinguishable p21 is constitutively expressed at low levels in normal cells of many vertebrate species⁵. Thus, p21 is a member of the class of normal cellular proteins whose coding sequences have been incorporated into the RNA genomes of various transforming retroviruses, such as Rous sarcoma virus⁶, Abelson murine leukaemia virus⁷ and feline sarcoma virus⁸. The phosphoproteins derived from these normal cellular proteins are required for transformation by their respective viruses and are ATP-using protein kinases⁹⁻¹¹. Viral p21 differs from the other kinases in that it specifically binds guanine-containing nucleotides and can transfer the γ -phosphate of GTP to a threonine residue on p21 (ref. 12).

The nucleic acid sequences of Ha- and Ki-MuSV are derived from both murine leukaemia virus and rat cellular DNA^{13,14}. In the 5.5-kilobase (kb) RNA genome of Ha-MuSV, which has been analysed in much greater detail than that of Ki-MuSV, the rat sequences (4.5 kb) have been divided into two components: (1) 1.0 kb in the 5' half of the genome representing the viral transforming (*src*) and p21-encoding gene¹⁵; (2) 3.5 kb derived largely, if not exclusively, from replication-defective, non-transforming, retrovirus-like sequences which are highly reiterated in rat DNA and which express themselves in some rat cells as '30S' RNA^{16,17}. Recently, using a molecularly cloned probe from the Ha-MuSV p21 *src* sequences, we have cloned two related cellular genes (*sarc*) from the rat¹⁸. These p21 *sarc* genes are distinct from each other in that one is colinear with the viral *src* sequences while the other has three introns in its coding sequence; nevertheless, with appropriate *in vitro* molecular reconstructions, both genes can induce cellular transformation mediated by high-level expression of strongly related, yet distinguishable, p21 *sarc* proteins¹⁸.

The gross structure of Ki-MuSV is very similar to that of Ha-MuSV. The murine leukaemia virus components of these two viruses are highly related and their rat cellular components show extensive homology^{13,14}. However, RNA heteroduplex analysis has revealed segments of rat sequences in the 5' half of

the two genomes which show little homology¹⁴. Comparison of these heteroduplexing data with our recently published data suggests that this segment of poor homology in Ha-MuSV corresponds to the 1.0-kb *src* sequence which encodes p21. These results were quite striking as the p21s encoded by Ha-MuSV and Ki-MuSV had shown only minor serological and electrophoretic differences¹⁹, and we had not anticipated an apparently larger divergence in their nucleic acid coding sequences.

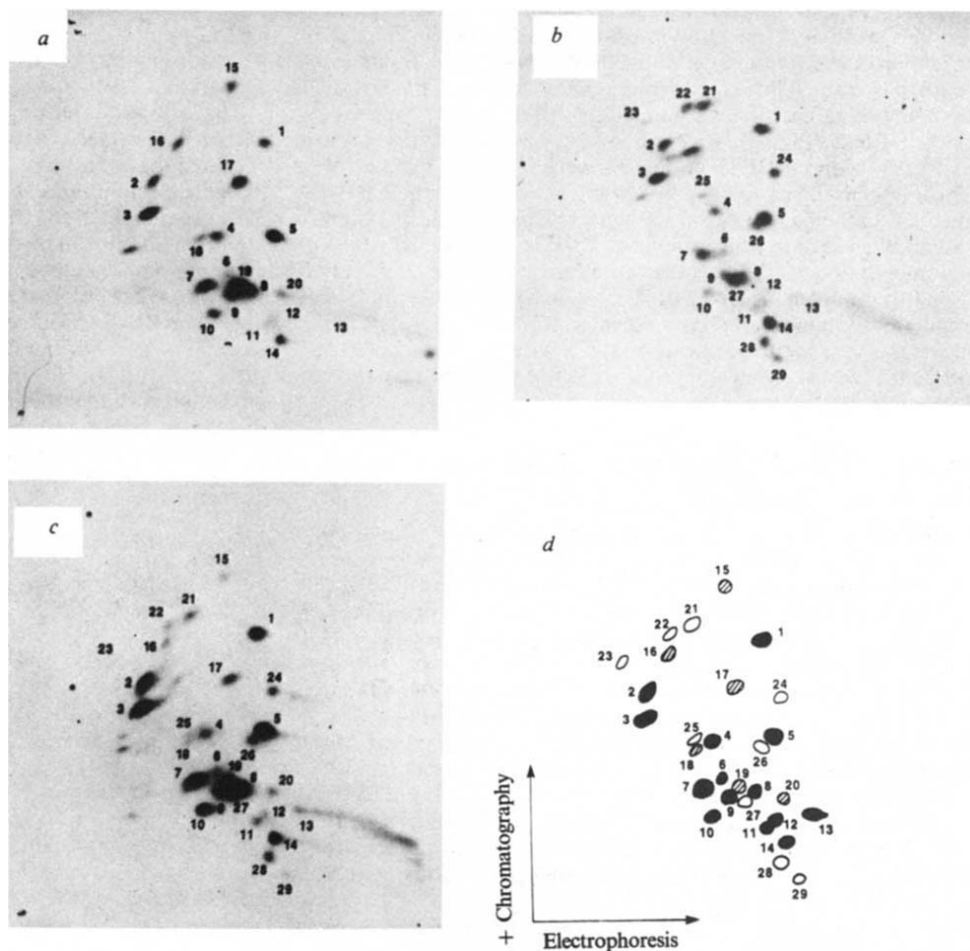
We have now analysed in greater detail the relationship between the p21s of Ha-MuSV and Ki-MuSV, and we have performed a more detailed structural comparison of the *src* genes of Ha-MuSV and Ki-MuSV using molecular clones of each virus. We have found that there is only a short segment [0.3 kilobase pairs (kbp)] of incomplete homology between their non-30S (that is *src*) components. Furthermore, using cloned fragments of each *src* sequence as radiolabelled probes for Southern blots of cellular DNA from a variety of species, we have shown that cellular *sarc* genes corresponding to Ha-*src* and Ki-*src* are detected as conserved genes in the DNA of several

Table 1 Infectivity of viral DNA clones

DNA clones	Treatment	Foci per μ g of viral DNA
H-1	Intact in pBR322	6.0×10^3
p-14	Self-ligation	0
HB-11	Self-ligation	(7)
KBE-2	Self-ligation	(5)
p-14 + HB-11	Ligation	9.0×10^2
p-14 + KBE-2	Ligation	3.1×10^2

The infectivity of the DNA preparations was tested through transfection onto NIH 3T3 cells using the calcium precipitation procedure^{22,24}. Clone H-1 DNA was used as a positive control. All other DNA clones were purified free of their pBR322 vectors by restriction endonuclease digestion (using enzymes from Bethesda Research Laboratories and New England Biolabs according to the suppliers' specification), agarose gel electrophoresis and electroelution²⁵. Purified inserts were ligated using T4 DNA ligase (New England Biolabs). DNA preparations (0.05–0.40 μ g per dish) were transfected using calf thymus DNA as carrier (25 μ g ml⁻¹), and foci of transformed cells were quantified from duplicate dishes. Representative foci were removed from the dishes using a cloning cylinder, so that the viral aetiology of the cellular transformations could be tested by p21 immunoprecipitation (see Fig. 5 legend for experimental details). The foci in parentheses are discussed in the text. Although these data are not related directly to the location of the viral *src* genes, we observed that HB-11 and KBE-2 DNA reproducibly induced low numbers of foci in these assays. However, when the DNA from such foci was analysed by Southern blotting, no new bands were detected homologous to the HB-11 or KBE-2 DNAs, and none of these foci expressed enhanced levels of p21. We do not understand the origin of these 'background' foci.

Fig. 1 Tryptic peptide analysis of p21 from cells transformed by Ha-MuSV or Ki-MuSV. MDCK dog cells transformed by Ha-MuSV, and C127 mouse cells transformed by Ki-MuSV, each in 60-mm dishes, were labelled for 22 h at 37 °C with 2 ml of lysine/arginine-free Dulbecco-Vogt modified minimal essential medium supplemented with 2% dialysed fetal calf serum and 125 μ Ci each of 14 C-lysine and 14 C-arginine (Amersham, 350 mCi mmol $^{-1}$). Cells were lysed with 2 ml buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM MgCl $_2$, 100 mM NaCl and 1% Triton X-100. After centrifugation for 30 min at 150,000 g, the cleared lysates were immunoprecipitated by antisera containing antibodies to Ha-MuSV p21 from tumour-bearing rats as described previously^{4,19}. The antigen-antibody complexes were precipitated by *Staphylococcus aureus* containing protein A. The precipitated proteins were analysed by discontinuous polyacrylamide gel electrophoresis in 0.1% SDS and were visualized by fluorography. The protein bands from the polyacrylamide gels, fixed without diphenyloxazole, were excised for tryptic peptide analysis. Gel slices were swelled and digested in 1 ml of 0.1 M NH $_4$ HCO $_3$ containing 20 g of DCC-treated trypsin (Sigma, Type X1) at 37 °C for 16 h. 20 μ g of fresh trypsin were added and incubation continued for another 8 h. The eluted tryptic peptides were lyophilized twice and finger-printed by electrophoresis for 70 min at 400 V in 30% formic acid on cellulose thin-layer plates (Schleicher and Schuell) followed by ascending chromatography in buffer containing *n*-butanol, acetic acid, pyridine and water (15:3:10:12 v/v). Peptide spots were visualized by fluorography. *a*, Ha-MuSV p21; *b*, Ki-MuSV p21; *c*, Ha-MuSV p21 mixed with equal c.p.m. of Ki-MuSV p21; *d*, interpretative sketching of *c*, where Ha-MuSV-specific, Ki-MuSV-specific, and common tryptic peptides are indicated by hatched, open and solid circles respectively.



vertebrate species. However, the individually conserved *Ha-sarc* and *Ki-sarc* gene(s) in each species are markedly divergent from one another, being present on different restriction fragments of DNA from that species.

Tryptic peptide analysis of Ha-MuSV and Ki-MuSV p21s

Our laboratory has demonstrated the close relationship between the Ha-MuSV and Ki-MuSV p21s by the criteria of serological cross-reactivity, electrophoretic mobility and guanine nucleotide-binding properties. To assess further, by an independent parameter, the relatedness between these *src* proteins and, indirectly, their genetic sequences, we performed two-dimensional tryptic peptide analysis on the p21s of Ha- and Ki-MuSV. To visualize every tryptic peptide except the most C-terminal one, virally transformed cells were labelled with 14 C-lysine plus 14 C-arginine. As shown in Fig. 1, 29 peptides have been resolved of which 6 are unique to Ha-MuSV p21, 9 are unique to Ki-MuSV p21, and 14 are shared by the two. Therefore, approximately two-thirds of Ki-MuSV and Ha-MuSV p21 peptides are shared, and this close, yet divergent, structural relationship has formed the basis for a detailed comparison of the genes encoding each viral p21.

Biological relationship between Ha- and Ki-MuSV molecular clones

Before studying the Ki-MuSV *src* sequences in detail, we sought biological evidence for the location of the Ki-MuSV *src* sequences relative to the well characterized Ha-MuSV *src*

region. The molecular cloning of the genomes of Ha-MuSV and Ki-MuSV has been reported. These clones, referred to as clone H-1 and clone 4 respectively^{20,21} (Fig. 2), were derived from unintegrated closed circular DNA molecules isolated shortly after cell infection by the viruses. Consequently, the cloned sequences are circularly permuted with respect to their linear viral genomes. As our initial step towards analysing the Ki-MuSV *src* sequences, we investigated whether these transforming sequences indeed were in a position analogous to their location in Ha-MuSV, as the earlier data had suggested. An appropriate direct biological assay would be the testing of the transforming activity of subgenomic Ki-MuSV fragments in the calcium phosphate precipitation-mediated DNA transfection assay²². An initial problem in this determination was the inability of viral *src* sequences alone to induce efficiently formation of foci of transformed cells. The viral long terminal repeat (LTR) sequences, highlighted as a solid box on clone H-1 in Fig. 2, prove useful in overcoming this problem. The LTR, presumably containing an efficient promoter of viral RNA transcription, is an amalgam of sequences from both the 5' and 3' termini of the RNA genome, which are juxtaposed by transfer of nascent DNA between templates during reverse transcription²³. Ligation of LTR DNA to both the transforming sequences of Ha-MuSV²⁴ and Moloney-MuSV²⁵, as well as the cellular *src* sequences related to these two viruses^{18,26}, has resulted in enhanced transforming activity mediated by these DNAs. Accordingly, a large subgenomic Ha-MuSV fragment containing the LTR was cloned in pBR322 and designated clone p-14 (Fig. 2). The derivation of Ha-MuSV clone HB-11, which contains the entire viral *src* sequence, has been described¹⁸.

Ligation of these two clones at their common *Bam*HI terminus resulted in efficient induction of transformed cell foci (Table 1), as expected. Therefore, a subgenomic Ki-MuSV fragment, residing in clone 4 in an analogous position to that of the HB-11 *src* clone in clone H-1, was cloned in pBR322 and designated clone KBE-2 (Fig. 2). Ligation of Ha-MuSV clone p-14 to Ki-MuSV clone KBE-2 at the *Bam*HI terminus resulted in efficient focus formation (Table 1), confirming that clone KBE-2 did contain the Ki-MuSV *src* sequences. This result is consistent with published data which implicate the 5' half of the Ki-MuSV genome as required for transformation²¹. As expected, cells from foci induced by HB-11 or KBE-2 DNA ligated to the LTR fragment contained markedly elevated levels of p21 (data not shown). These results indicate that the viral p21 *src* genes of Ha-MuSV and Ki-MuSV are located in the same region of each respective viral genome.

Fine structural comparison of Ki- and Ha-MuSV *src* genes

Having confirmed the general location of the *src* sequences within the Ki-MuSV genome, we then compared these sequences with their Ha-MuSV counterparts. Clones KBE-2 and HB-11 were hybridized for heteroduplex analysis (Fig. 3, *d-f*). The 1.1-kbp segment '11+8+12' (Fig. 3*f*) in clone HB-11 represents precisely the published location and size of the Ha-MuSV *src* sequences relative to the 5'-terminal *Bam*HI site in the clone^{15,27}. Similarly, segments '6' and '10' (Fig. 3*f*) in clone HB-11 coincide with the reported location of rat 30S sequences. Heteroduplexing of clone KBE-2 to the rat 30S clone shows that segments '1' + '3' (Fig. 3*c*) correspond to the rat 30S sequences and segment '2' represents sequences specific to Ki-MuSV. Segments '7-9' in Ki-MuSV DNA (Fig. 3*f*) correspond to the Ki-specific segment '2' in Fig. 3*c*, and segment '8'

in Fig. 3*f* is homologous to a portion of the Ha-MuSV *src* region. Therefore, a 1.75-kbp segment in Ki-MuSV DNA, corresponding to segment '2' in Fig. 3*c*, contains the Ki-MuSV *src* sequences.

These heteroduplexing data have confirmed and extended the earlier RNA-DNA heteroduplexing experiments¹⁴ and have specified the location of *src* within Ki-MuSV DNA by virtue of its partial homology to Ha-MuSV *src*. In particular, our measurements of the heteroduplexes between these cloned DNA fragments can be combined with restriction endonuclease mapping data to clone a Ki-MuSV-specific fragment. To this end, we have developed a detailed restriction endonuclease map of clone KBE-2 and have compared it with the published map of clone HB-11, with the heteroduplexing data superimposed on the maps (Fig. 4). Note several features in this comparison: (1) virtually every site in the rat 30S DNA sequences (open box) of the clones is present in both Ha-MuSV and Ki-MuSV; (2) there is a complete site mismatch in the respective *src* sequences; (3) the location on clone HB-11 of the Ha-MuSV-specific (that is,

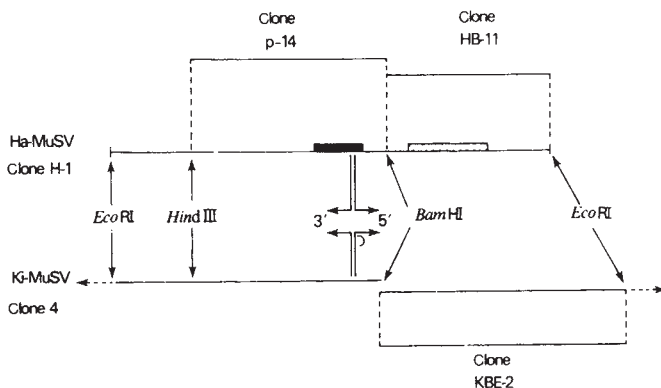


Fig. 2 Structural relationships among the viral clones. Ha-MuSV and Ki-MuSV RNA genomes had been molecularly cloned from preintegration circular DNA forms. Advantage was taken of the fact that retroviral RNA is reverse-transcribed after cellular infection into closed circular double-stranded DNA. Based on the uniqueness of particular restriction endonuclease sites, unintegrated viral DNA was purified from the Hirt supernatant of infected cells, cleaved with *Eco*RI (Ha-MuSV) or *Bam*HI (Ki-MuSV), and cloned at these respective restriction sites in pBR322. (Ha-MuSV DNA originally had been cloned in λ gtWES $\cdot$ λ B³⁶ but subsequently was recloned in pBR322.) Consequently, Ha-MuSV clone H-1 and Ki-MuSV clone 4 are circularly permuted with respect to their RNA genomes, such that the termini of the genomes are juxtaposed in the DNA clones. The 5' and 3' halves of the viral genomes are demarcated as indicated on the DNA maps. The subgenomic fragments p-14, HB-11 and KBE-2 were derived from their respective parental clones by double digestion with the indicated restriction endonucleases, preparative gel electrophoresis and molecular cloning at the appropriate sites in pBR322. Clone p-14 contains the LTR sequences (solid box) which have a promoter-like sequence of the same 5'-3' polarity as the viral genome³⁷ and which significantly enhance the transformation efficiency of viral *src* genes. Clone HB-11 (ref. 18) contains the Ha-MuSV transforming sequences (cross-hatched box).

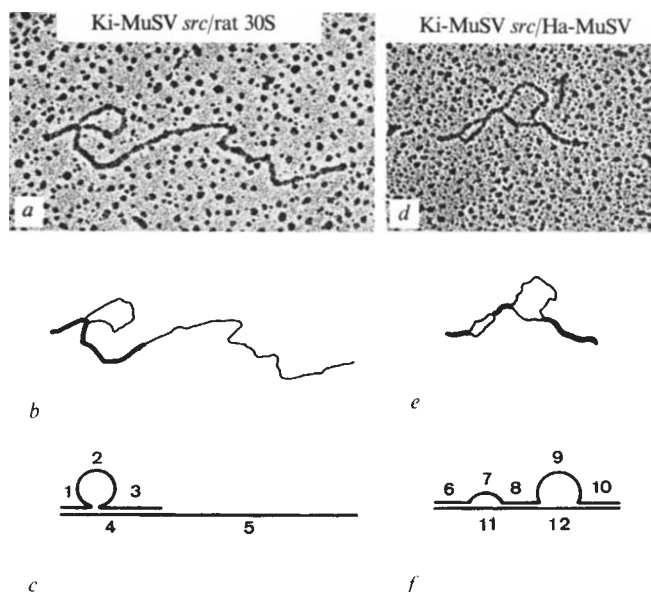


Fig. 3 Heteroduplex analyses of Ki-MuSV *src*, Ha-MuSV *src* and rat 30S DNA molecules. Heteroduplexes were prepared as described previously^{27,38}. Briefly, a mixture of linear DNA molecules (each at a concentration of 4–6 μ g ml⁻¹), freed from their cloning vectors by restriction endonuclease digestion and preparative gel electrophoresis, was denatured by incubation in 0.1 M NaOH for 10 min at 22 °C. The solution was neutralized by the addition of 1/5 volume of 1 M Tris-HCl, pH 7.0. Deionized formamide was added to a final concentration of 50% and renaturation was permitted by incubation for 30 min at 22 °C. Heteroduplexes were mounted for electron microscopy after spreads on a hypophase of 18% formamide. The lengths of single-stranded Φ X174 (5,375 bp) and double-stranded SV40 (5,240 bp) circular DNA molecules were determined in separate experiments in similar spreading conditions to obtain a conversion factor for obtaining measurements of single- and double-stranded regions of the heteroduplexes. We calculate from these experiments that 1 μ m contour length of double-stranded DNA = 3,080 bp, 1 μ m single-stranded DNA = 3,040 bp. Electron micrographs ($\times$ 54,000) are *a*, heteroduplex of Ki-MuSV fragment (clone KBE-2) and rat 30S DNA (clone 27A, which was cloned at the *Sac*I site in λ gtWES $\cdot$ λ B²⁷) and *d*, heteroduplex of Ki-MuSV (clone KBE-2) and Ha-MuSV (clone HB-11) fragments. Interpretative tracings of the heteroduplexes are shown in *b* and *e*. Schematic representations (*c*, *f*) are based on the measurements of > 15 molecules. Contour lengths (in kb) were as follows: 1 = 0.40 $\pm$ 0.02, 2 = 1.75 $\pm$ 0.10, 3 = 1.02 $\pm$ 0.06, 1–3 = Ki-MuSV clone, 2 = Ki-MuSV *src*, 4 = 1.50 $\pm$ 0.08, 5 = 3.83 $\pm$ 0.33, 4–5 = rat 30S, 6 = 0.27 $\pm$ 0.03, 7 = 0.52 $\pm$ 0.06, 8 = 0.34 $\pm$ 0.08, 9 = 1.18 $\pm$ 0.14, 10 = 0.71 $\pm$ 0.05, 11 = 0.33 $\pm$ 0.07, 12 = 0.44 $\pm$ 0.11, 6–10 = Ki-MuSV clone, 7–9 = Ki-MuSV *src*, 6+8+10+11+12 = Ha-MuSV clone, 11+8+12 = Ha-MuSV *src*.

free of rat 30S) subgenomic fragment BS-9 (ref. 15) approximately circumscribes the region of cross-homology between Ha- and Ki-MuSV *src* (solid box).

Pursuing this latter point, we performed Southern blot hybridization with 32 P-labelled BS-9 (Ha-specific) DNA as probe and were struck by its virtual lack of hybridization to clone KBE-2 (data not shown). We questioned whether this could be a function of differences in the relative stringency of hybridization conditions in the heteroduplexing and the Southern blotting procedures. Using the recently described formamide hybridization conditions which enable Southern blots to be hybridized under various levels of stringency²⁸, we found that 32 P-BS-9 and Ki-MuSV *src* hybridized well only in 'non-stringent' conditions (data not shown). This lack of reactivity of the two *src* sequences in stringent conditions suggests that a Ki-MuSV-specific probe might be isolated free not only of 30S RNA sequences but also of Ha-MuSV cross-reactivity in stringent conditions.

Detection of Ki-MuSV as distinct from Ha-MuSV *src* sequences in cellular DNA

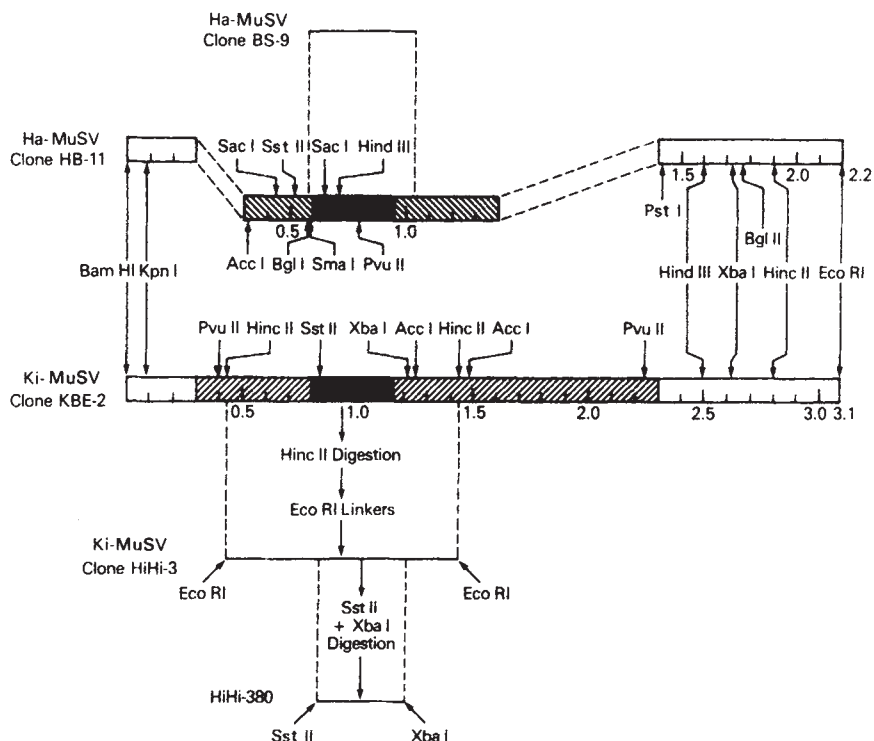
Our approach to the development of a Ki-MuSV *src* probe depended on the probe being completely free of rat 30S sequences, insofar as these genes related to 30S are highly reiterated in rats and, to a lesser extent, in mice. Therefore, based on its lack of hybridization to cloned rat 30S DNA, a 1.0-kbp fragment of clone KBE-2 DNA was cloned in pBR322 (clone HiHi-3, Fig. 4). For use as a Ki-MuSV *src*-specific probe, a fragment of clone HiHi-3 was purified by preparative gel electrophoresis (HiHi-380, Fig. 4). We then used both the Ha-specific and Ki-specific DNAs as radiolabelled probes for Southern blots of high-molecular-weight DNA from cells of various species (Fig. 5). As biological controls for the specificity of the *src* probes, we performed hybridizations to *Eco*RI-digested DNAs from NIH 3T3 mouse cells infected with the

various rat-derived sarcoma viruses. The Ha-specific probe, hybridized to *Eco*RI-digested DNA from cells non-productively transformed by Ha-MuSV, detected an additional band compared with control cell DNA which was not detected by the Ki-specific probe (data not shown). Conversely, the Ki-specific probe, but not the Ha-specific probe, detected an extra band in *Eco*RI-digested DNA from cells non-productively transformed by Ki-MuSV (Fig. 5a and b, lanes 6).

A third cell line studied was NIH 3T3 cells transformed by the rat sarcoma virus (RaSV). This virus, whose entire genome is rat derived, was isolated after *in vitro* co-cultivation of two lines of rat cells²⁹. The only known gene product of RaSV is a 29,000-MW protein³⁰ which is closely related to Ki- and Ha-MuSV p21s in terms of common methionine-containing tryptic peptides as well as serological cross-reactivity³¹. When blots of *Eco*RI-digested DNA from RaSV-transformed NIH 3T3 cells were hybridized with the Ha-MuSV and Ki-MuSV *src* probes, an extra band relative to control cells was detected only with the Ha-specific probe, not the Ki-specific probe (Fig. 5a and b, lanes 5). These results demonstrate that the RaSV transforming sequences are more closely related to those of Ha-MuSV than to those of Ki-MuSV.

Having confirmed the specificity of these *src* probes, we then tested DNAs of rat, chicken, human and mouse for cellular p21 *src* sequences related to the viral *src* probes. It can be seen that the Ha-specific probe detected one or two bands in each of these *Eco*RI-digested DNAs (Fig. 5a, lanes 1-4). The Ki-specific probe detected one to three bands (Fig. 5b, lanes 1-4) distinctly different from the corresponding Ha-specific bands. The exceedingly weak hybridization of the Ki-specific probe to the 2.1- and 4.2-kbp chicken DNA fragments (Fig. 5b, lane 2) suggests that the normal cellular *src* sequences related to Ki-MuSV, having diverged more rapidly in chickens, are not as broadly conserved evolutionarily as are the Ha-MuSV *src* sequences.

Fig 4 Detailed structure of Ha-MuSV and Ki-MuSV transforming sequences. A detailed restriction endonuclease map, on a scale demarcated in kbp, was developed for Ki-MuSV clone KBE-2. The detailed map of Ha-MuSV clone HB-11 is reproduced from the published map of the complete Ha-MuSV genome clone H-1¹⁵. Superimposed on these maps are the heteroduplex data of Fig. 3. The open box represents common rat 30S RNA sequences; the solid box represents sequences shared by Ha-MuSV and Ki-MuSV but not rat 30S RNA; the hatched boxes represent sequences unique to either Ha-MuSV (left-hatched) or Ki-MuSV (right-hatched). Note the discontinuity of scale on clone HB-11 (dotted lines), drawn to compensate for the size differential between the Ha-MuSV- and Ki-MuSV-specific (hatched) sequences. The details of the derivation of Ha-MuSV clone BS-9 have been published elsewhere¹⁵. To derive cloned sequences as a specific probe for Ki-MuSV, we sought a fragment of clone KBE-2 which would not hybridize to rat 30S RNA sequences. Accordingly, singly and doubly restricted fragments of clone KBE-2 were electrophoresed, blotted and hybridized³⁶ to 32 P-labelled clone 27A DNA. A *Hinc*II-*Hinc*II fragment (map positions 0.4-1.4) was purified by preparative gel electrophoresis, and after blunt-end ligation of *Eco*RI linkers (Collaborative Research) to the DNA, molecules were digested with *Eco*RI, purified free of linker monomers by Sepharose 2B (Pharmacia) chromatography and cloned at the *Eco*RI site of pBR322. This clone is designated Ki-MuSV clone HiHi-3. When it was used as a radiolabelled probe for Southern blots of cellular DNA sequences, innumerable bands were detected in rat DNA, even though one to three bands were detected in DNA from other species (data not shown). The origin of this redundancy with respect to rat DNA was unclear, although it could have represented rat 30S sequences present in clone HiHi-3 yet missing in the rat 30S clone which we had used to define HiHi-3. Nevertheless, we sought a fragment of clone HiHi-3, which would be 'single copy' in rat DNA, by trimming the clone HiHi-3 insert. To prepare such a fragment, the clone HiHi-3 viral insert was digested with *Sst*II and the 0.6-kbp fragment purified by preparative gel electrophoresis. This fragment was digested with *Xba*I, then the 0.38 kbp fragment (HiHi-380) was purified by preparative gel electrophoresis.



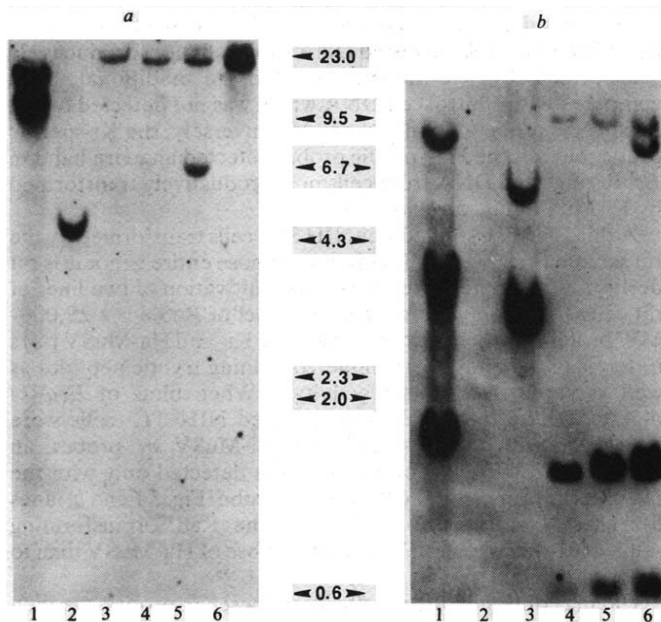


Fig. 5 Southern blot analysis of restriction endonuclease-digested high-molecular-weight cellular DNA. 50 µg of cellular DNA, prepared by established procedures³⁹, were digested for 16 h with 100 units *Eco*RI, electrophoresed, blotted and hybridized (in the 'stringent' conditions described in Fig. 4 legend) to either of two ³²P-labelled DNA probes (1.5 × 10⁷ c.p.m.). *a*, ³²P-labelled BS-9 (Ha-specific) DNA. *b*, ³²P-labelled HiHi-380 (Ki-specific) DNA. Sources of high-molecular-weight DNA were: 1, FRE rat cells; 2, chicken cells; 3, human cells; 4, uninfected NIH 3T3 cells; 5, NIH 3T3 cells transformed by the rat sarcoma virus; 6, NIH 3T3 cells transformed by Ki-MuSV. The arrows indicate the positions of ³²P-labelled λ/*Hind*III DNA markers (length in kbp). Panel *a*, lane 1 portrays the two rat cellular *sarc* genes related to Ha-MuSV *sarc*, as has been reported elsewhere¹⁸. The hybridizing bands represent the intron-containing (20 kbp) and colinear (13 kbp) forms of Ha-*sarc*.

Discussion

The relationship between Ha-MuSV and Ki-MuSV is a more complex one than had been thought previously. Minor serological and electrophoretic differences between the viral p21s had suggested that the respective *sarc* proteins were merely variants of one another and were derived from the same cellular p21 *sarc* gene. This conclusion was consistent with the ability of these viruses to induce similar diseases in rodents and morphologically similar cellular transformants *in vitro*. The data presented here clearly demonstrate that the Ha- and Ki-MuSV *sarc* sequences are derived from distinct cellular p21 *sarc* genes. On the basis of the heteroduplexing, hybridization and tryptic peptide data, we deduce that there are two domains within the genes coding for the respective viral p21 molecules. One of these domains represents sequences unique to each p21 gene, sequences that are a subset of the hatched region of the viral genomes in Fig. 4. The other domain represents sequences shared by both the p21 molecules, as indicated by the solid box in Fig. 4. Assuming that these common sequences are entirely coding sequences, their length (0.35 kbp) would contain the genetic information for ~11,000 daltons of polypeptide chain, that is, one-half of the p21 polypeptide. Moreover, the ability of ³²P-Ha-specific DNA to hybridize to Ki-MuSV *sarc* in non-stringent but not in stringent conditions suggests that the Ki-common and Ha-common sequences are approximately 10–15% divergent. Most of this divergence would involve 'wobble-codon' changes and other nucleotide changes resulting in conservative amino acid substitutions, such that >50% of the tryptic peptides remain shared between the two viral p21s. It seems likely that these common sequences account for the serological cross-reactivity, guanine nucleotide-binding capacity and common biological potential

(transformation) of the viral p21s. Apparently this represents the selection pressure for the conservation of the p21 amino acid sequences during the evolution of Ha-MuSV and Ki-MuSV p21 genes. Furthermore, our preliminary nucleotide sequence analysis of Ha-MuSV places these common sequences in the 5' half of the polypeptide chain (unpublished data). At this point, we can only speculate that the sequences unique to either Ha- or Ki-MuSV p21 contribute to as yet undiagnosed differences between the proteins.

Our laboratory has demonstrated the expression of low (relative to cells transformed by Ha- or Ki-MuSV) levels of p21 in normal cells from a wide variety of species, and our Southern blots of various cellular DNAs have shown the broad evolutionary conservation of both Ha- and Ki-MuSV cellular genes. Given the presence of multiple related, yet distinct genes, are all or only some of the cellular genes being expressed in different cells? We have shown that rat DNA has two genes homologous to Ha-MuSV *sarc*: one is apparently colinear with p21 *sarc* while the other has three introns in its coding sequence (it is likely that one of these genes is the progenitor of the putatively transformation-specific p29 of the third rat-derived sarcoma virus RaSV). Both genes are fully functional, as each is capable of causing cellular transformation after DNA transfection. These transfections also have demonstrated that elevated levels of a normal cellular protein are sufficient to cause cellular transformation. With respect to homology to Ki-MuSV *sarc*, blots of *Eco*RI-digested rat DNA have three prominent bands, while blots of rat DNA digested with other restriction endonucleases have two to three major bands (unpublished data). These data suggest that there may be two Ki-MuSV cellular *sarc* genes in the rat, thereby making a minimum total of four rat genes capable of encoding a constitutively expressed p21. As there has probably been selection pressure favouring the retention of this entire family of p21 genes, it would be important to determine which of the genes is expressed in different rat cells. With respect to mouse cells, we have recently reported that a line of undifferentiated haematopoietic cells expresses higher levels of p21 than do Ha-MuSV or Ki-MuSV-transformed cells³², and we wish to determine which gene is encoding this p21. The structures of the various cellular and viral p21 polypeptides and their *in vivo* expression should elucidate functional differences among the various p21s. For example, there may be a tissue- or differentiation-specific pattern of expression among the genes, as in the globin gene family³³. By analogy to fetal and adult forms of globin, each distinct p21 might possess unique biochemical properties pivotal to its normal differentiation-related physiological role.

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- Harvey, J. J. *Nature* **204**, 1104–1105 (1964).
- Kirsten, W. H. & Mayer, L. A. *J. natn. Cancer Inst.* **39**, 311–335 (1967).
- Scher, C., Scolnick, E. M. & Siegel, D. *Nature* **256**, 225–227 (1975).
- Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. *J. Virol.* **31**, 546–556 (1979).
- Langbeheim, H., Shih, T. Y. & Scolnick, E. M. *Virology* **106**, 292–300 (1980).
- Spector, D. H., Varmus, H. E. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4102–4106 (1978).
- Witte, O. N., Rosenberg, N. & Baltimore, D. *Nature* **281**, 396–398 (1979).
- Sherr, C. J., Fedele, L. A., Donner, L. & Turek, L. P. *J. Virol.* **32**, 860–875 (1979).
- Collett, M. S. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2021–2024 (1978).
- Witte, O. N., Dasgupta, A. & Baltimore, D. *Nature* **283**, 826–831 (1980).
- Van de Ven, W. J. M., Reynolds, F. H. & Stephenson, J. R. *Virology* **101**, 185–197 (1980).
- Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weeks, M. O. & Scolnick, E. M. *Nature* **287**, 686–691 (1980).
- Shih, T. Y. *et al. J. Virol.* **27**, 45–55 (1978).
- Chien, Y. H. *et al. J. Virol.* **31**, 752–760 (1979).
- Ellis, R. W. *et al. J. Virol.* **36**, 408–420 (1980).
- Scolnick, E. M., Vass, W. C., Howk, R. S. & Duesberg, P. H. *J. Virol.* **29**, 964–972 (1979).
- Tsuchida, N., Gilden, R. & Hatanaka, M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4503–4507 (1974).
- DeFeo, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 3328–3332 (1981).
- Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. *Virology* **96**, 64–79 (1979).
- Chang, E. H. *et al. J. Virol.* **35**, 76–92 (1980).
- Tsuchida, N. & Vesugi, S. *J. Virol.* **38**, 720–727 (1981).
- Graham, F. L. & van der Eb, A. J. *Virology* **52**, 456–461 (1973).
- Gilboa, E. *et al. Cell* **16**, 863–874 (1979).
- Chang, E. H., Ellis, R. W., Scolnick, E. M. & Lowy, D. R. *Science* **210**, 1249–1251 (1980).

25. Blair, D. G., McClements, W. L., Oskarsson, M. K., Fischinger, P. J. & Vande Woude, G. F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3504–3508 (1980).
26. McClements, W. L. *et al. Cold Spring Harb. Symp. quant. Biol.* **45** (in the press).
27. Young, H. A. *et al. Virology* **107**, 89–99 (1980).
28. Howley, P. M., Israel, M. A., Law, M.-F. & Martin, M. A. *J. biol. Chem.* **254**, 4876–4883 (1979).
29. Rasheed, S., Gardner, M. B. & Huebner, R. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2972–2976 (1978).
30. Young, H. A., Shih, T. Y., Scolnick, E. M., Rasheed, S. & Gardner, M. B. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3523–3527 (1979).
31. Young, H. A. *et al. J. Virol.* **38**, 286–293 (1981).
32. Scolnick, E. M., Weeks, M. O., Shih, T. Y., Ruscetti, S. K. & Dexter, T. M. *Molec. cell. Biol.* **1**, 66–74 (1981).
33. Nienhuis, A. W. & Bernz, E. J. *New Engl. J. Med.* **297**, 1318–1328, 1371–1381, 1430–1436 (1977).
34. Lowy, D. R., Rands, E. & Scolnick, E. M. *J. Virol.* **26**, 291–298 (1978).
35. McDonnell, M. W., Simon, M. N. & Studier, F. W. *J. molec. Biol.* **110**, 119–146 (1977).
36. Hager, G. L. *et al. J. Virol.* **31**, 795–809 (1979).
37. Dhar, R., McClements, W. L., Enquist, L. W. & Vande Woude, G. F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3937–3941 (1980).
38. Davis, R. W., Simon, M. & Davidson, N. *Meth. Enzym.* **210**, 413–428 (1971).
39. Gross-Bellard, M., Oudet, P. & Chambon, P. *Eur. J. Biochem.* **36**, 32–38 (1973).

A reinvestigation of the role of the grey crescent in axis formation in *Xenopus laevis*

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Gravitationally induced displacements of the contents of the frog egg can predictably determine the orientation of the subsequent dorsal–ventral axis of the embryo, regardless of the original position of sperm entry or of the grey crescent. In certain conditions, these displacements in the egg can also lead to the formation of a second axis, that is, to twinning. The previously reported ability of grafts of grey crescent cortex to induce secondary axes in recipient eggs is interpreted here as an unrecognized twinning effect of gravity. Our results lead us to question the classic interpretation of the grey crescent as a dorsal determinant in amphibian development.

The unfertilized egg of most frogs and salamanders is organized with radial symmetry about its animal–vegetal axis. The embryo, however, has clear bilateral symmetry organized along anterior–posterior and dorsal–ventral axes. Early embryologists recognized the following events in the establishment of the dorsal–ventral axis^{1–5}: (1) a single sperm randomly enters the animal hemisphere, giving the egg its first bilateral symmetry; (2) before first cleavage, the egg forms a grey crescent, a region of reduced pigmentation in the animal hemisphere which, in the case of the frog egg, is opposite the side of sperm entry; (3) at gastrulation, about 15 cleavages later, the blastopore appears first at the lower limit of the surface region previously occupied by the grey crescent; and (4) dorsal structures of the embryo originate from the grey crescent region of the egg. As reported by Spemann and Mangold^{5,6}, the gastrula contains in the dorsal blastopore lip a group of cells which induce neighbouring cells to undergo dorsal development and are themselves incorporated into dorsal structures. Thus, the dorsal regions of the embryonic axis owe their location to the position of these inducer cells, the Spemann organizer, while ventral development occurs where the organizer is absent.

As the Spemann organizer region is so clearly responsible for the ‘dorsalization’ of the gastrula and neurula, the grey crescent, its topographical precursor, has been ascribed the capacity to generate the organizer. The most detailed proposal for the grey crescent’s role is found in the cortical field hypothesis of Dalcq and Pasteels^{1,7}: the developmental fate of each egg region is said to derive from local values of two kinds of quantitative material gradients, the first a cortical gradient centred in the grey crescent at the egg surface, and the second, an internal cytoplasmic gradient oriented in the vegetal to animal direction following the concentration of yolk platelets. The blastopore and organizer would arise much later at the site where the combined value of the gradients is greatest. The two gradients were said to be distinguishable experimentally by their response to gravity or centrifugation, since the yolk platelets, but not the cortical materials, were expected to rearrange easily in the egg.

Although Pasteels¹ devised tests of this hypothesis involving rotation and inversion of eggs at the time of grey crescent formation, his results were rather ambiguous and have been generally neglected compared with the direct, dramatic support

of Curtis⁸, who reported that the dorsalizing activity of the grey crescent cortex was transplantable. He removed a piece of grey crescent cortex from a donor egg of *X. laevis* shortly before first cleavage and grafted it into the equatorial surface of the prospective ventral side of a recipient egg at or close to first cleavage. In all 11, eggs receiving cortex from the crescent region double embryonic axes developed, one supposedly from the resident crescent region and one from the transplanted crescent region. Thus, the grey crescent cortex was concluded to be a dorsalizing locus, which preserved its activity on transplantation.

More recently, Nieuwkoop and co-workers^{3,9,10} have obtained experimental results which contradict a strict cortical gradient hypothesis. They found that at the mid-blastula stage, the potential for dorsal development is carried by a group of cells in the vegetal hemisphere that are not topographically superimposable with the grey crescent. These vegetal cells induce the Spemann organizer to form in adjacent animal hemisphere cells, coincidentally in the grey crescent region. Furthermore, recent fate maps of *X. laevis* indicate that, contrary to earlier conclusions, the cells of the Spemann organizer do not derive from the grey crescent cortical area of the egg but from deeper cytoplasmic regions^{11,12}. Because of these discrepancies, we have repeated and extended for *X. laevis* several of the classic experiments on which the importance of the grey crescent rests.

Topographical relationship of sperm entry, grey crescent and blastopore in *X. laevis*

Shortly after fertilization, a small amount of pigment accumulates around the sperm entry point (SEP) in the animal hemisphere, producing a dark spot¹³. Approximately mid-way in the period from fertilization to first cleavage, the grey crescent appears on the side of the animal hemisphere away from the SEP, as a region of lighter and more granular pigmentation¹³. In ~90% of eggs, the crescent forms in the sector 135°–180° from the SEP¹⁴, but the broadness and diffuseness of the crescent in *X. laevis* preclude more accurate measurement of its position relative to the SEP or blastopore. As the SEP can be very accurately localized, we have marked eggs with Nile blue at an equatorial position (that is, the boundary of the pigmented and

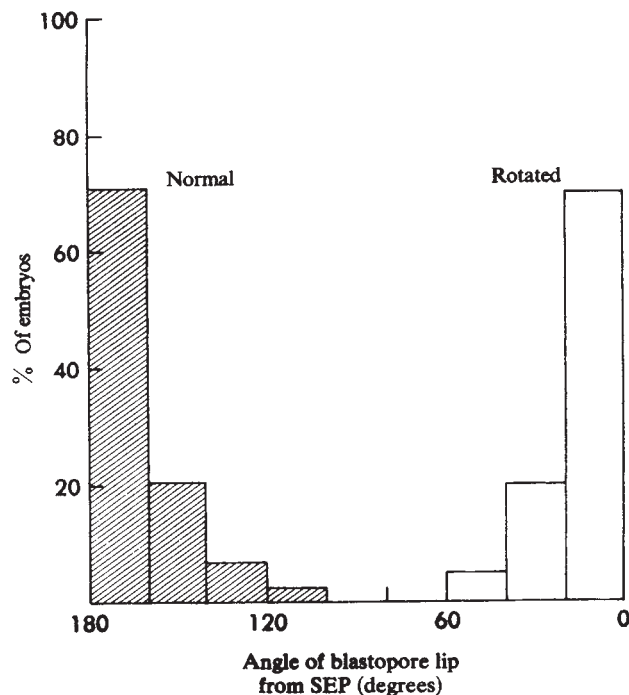


Fig. 1 The topographical relationship of the sperm entry point (SEP) to the dorsal blastopore lip in *X. laevis* embryos developing from normal and rotated eggs. Unfertilized eggs were squeezed from frogs into 100% MR (100 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 5 mM Na-HEPES, pH 7.6) and fertilized *in vitro* in 30% MR. After fertilization, eggs were dejellied with 2% cysteine hydrochloride, brought to pH 7.9 with NaOH, and washed extensively with 20% MR before storage until use. Procedures were as described elsewhere^{28,29}. Eggs were placed in 5% Ficoll-20% MR and were marked at the equatorial margin (pigmentation boundary) beneath the SEP by application of a small crystal of Nile red, a sparingly soluble form of Nile blue¹⁶, or by injection of 1% Nile blue in 20 mM sodium phosphate, pH 7.4. The rotated eggs were inclined 90° to the side, with the SEP and dye mark uppermost, starting 50 min after fertilization (0.55 on a normalized scale, 19°C), and continuing for 30 min, at which time the eggs and unrotated controls were transferred to 20% MR where the perivitelline space rehydrated and the eggs returned to gravitational alignment. After 9–15 h, when the blastopore lip first appeared, eggs were scored for the position of the lip relative to the dye mark, by inverting the egg and measuring the angle separating the lip and mark. The histogram places eggs in 20° angular spreads. Normal eggs, not rotated, cluster at the 180° angular separation, that is, the blastopore tends to be opposite the side of sperm entry. Rotated eggs group around a 0° separation, with the lip appearing on the same side as the SEP.

non-pigmented hemispheres) closest to the SEP and have recorded the position of the earliest blastopore lip 10 h later, at the gastrula stage. As shown in Fig. 1, in 70% of eggs, blastopores originate in the sector 160–180° from the dye mark (and hence the SEP of the egg), 27% in the sector 120–160° from the SEP, and 3% in the 100–120° sector. Thus, the SEP is a good but not perfect indicator of the blastopore position and the future dorsal-ventral axis of the embryo.

Dissociation of the topographical relations by gravity

We have repeated and extended with *X. laevis* eggs several of the experiments of Ancel and Vintemberger¹⁵ who demonstrated the strong control exerted by gravity on axis determination in eggs of *Rana fusca*. In our modification¹⁶ of their procedure, we have marked dejellied eggs with Nile blue at the equator near the SEP and immersed them in a solution of 5% Ficoll (Pharmacia), a hydrophilic sucrose polymer which dehydrates the perivitelline space and causes the fertilization envelope to collapse on to the egg surface. The egg can then be rotated 90° so that its animal-vegetal axis, which is normally vertical due to the dense yolk of the vegetal hemisphere, is then horizontal. In the Ficoll solution, the rotated egg is unable to return to the vertical orientation in the absence of lubrication from the perivitelline space. Inside the egg, the dense contents of

the vegetal hemisphere flow slowly downwards, driven by gravity, displacing upwards the less dense cytoplasm of the animal hemisphere^{1,15}. These rearrangements continue until the egg is returned to the vertical orientation manually or by transferring the egg to a solution without Ficoll, in which the perivitelline space rehydrates. With reference to the egg's original animal-vegetal axis, gravity causes a slight displacement of cytoplasmic contents towards one side of the egg.

The effect of brief horizontal orientation on axis determination is very striking. Shortly after fertilization, we turned eggs horizontally to a position with the SEP and blue mark uppermost, left them in this orientation at 20°C for 30 min and then allowed the eggs to develop in the normal orientation until the gastrula stage, when we scored for the position of the earliest blastopore. To our surprise, the blastopore originated directly beneath the blue mark, that is, on the same side of the egg where the sperm had entered. Brief rotation had completely reversed the egg's selection of a dorsal-ventral axis. These data are shown in Fig. 1 on the right side of the histogram: in 70% of eggs the blastopore initiated in the sector 0–20° from the mark, 20% in the 20–40° sector and 10% in 40–60° sector. From observations of the Nile blue mark in the neurula and tailbud stages, we confirmed that dorsal embryonic structures did indeed form from the SEP side of these eggs. We also rotated eggs to horizontal inclinations in which the SEP and dye mark were to the left or right side rather than uppermost, and found that whatever region of the egg equator is uppermost during the rotation period becomes the site of dorsal development in the embryo. Clearly, the egg has selected its dorsal-ventral axis based on its relationship to the gravitational field and not on the position of the SEP. Normally the egg, in its vertical orientation, is free to rotate in the perivitelline space, protected from such gravity-induced lateral rearrangements of its contents.

Figure 2 shows the effects of gravity on eggs at various times from fertilization to first cleavage, as seen using the Ficoll method. Control eggs are shown in the right-hand part of the graph; unrotated eggs gave blastopores approximately opposite (158°) the SEP, and eggs rotated horizontally with the SEP directed downwards gave a narrower angular distribution (173°) than the unrotated controls, as if rotation in that direction reinforces the normal sperm-directed selection of an axis.

In contrast to the controls, the experimental eggs gave clear evidence of axis reversal. As the extent of reversal depends on the time at which the rotation began, the experimental results can be considered in two parts: that is, the effects of gravity in the pre-grey crescent period (time 0.0–0.5) and in the post-grey crescent period (0.5–1.0), where the scale of 0 to 1.0 represents normalized time from fertilization to first cleavage (100 min at 18°C). The egg is very sensitive to horizontal orientation in the pre-crescent period and reversal is essentially complete. A 12-min period at 90° inclination is sufficient to reverse 100% of the eggs, and an angle of inclination of just 30°, rather than 90°, is fully effective (data not shown). Thus, the normal topographical relation of the SEP to the locus of prospective dorsal development in the egg can be easily disrupted in the pre-crescent period, with the egg using the gravitational perturbation for its selection of an axis, as was recognized by Ancel and Vintemberger¹⁵.

In the post-crescent period, the egg becomes more refractory to the effects of gravity. As shown in Fig. 2, a horizontal inclination starting after the time 0.5 is still effective in determining the axis, although the resistance of the egg to gravity increases between time 0.7 and 0.9 and is essentially complete by 1.0, when first cleavage begins. Pasteels¹ interpreted this resistance as a reflection of the establishment of the grey crescent which, as a cortical determinant, was by definition not perturbable by gravity. Our data do not really fit this interpretation because even at times 0.7 and 0.8, when the grey crescent is fully visible in *X. laevis* eggs, a significant fraction of the eggs are still affected by gravity. On the other hand, we do not know when grey crescent formation is complete in *X. laevis* eggs, and note that in other frog species the formation continues

Table 1 Effects of gravity and centrifugal force in the period after grey crescent formation and before first cleavage

Starting time (normalized)	Duration (min)	Force (g)	Angle of inclination of egg, SEP side uppermost	No. of eggs	Position of neural groove (average angle, in degrees from SEP)
(1) —	—	1	0	470	138
(2) 0.40	20	1	90	109	27
(3) 0.90	20	1	90	98	140
(4) 0.90	90	1	90	32	141
(5) 0.90	4	26	90	120	112
(6) 0.90	4+90	26+1	90	43	74
(7) 0.95	4	50	45	10	30
(8) 0.95	4	50	90	24	20

Eggs of *X. laevis* were fertilized, dejellied and embedded upright in 9% gelatin (175 Bloom, Sigma)/30% modified Ringer's solution^{28,29} in 35-mm plastic tissue culture dishes. The sperm entry side of each egg was oriented towards a marked side of the dish. The time of the start of gravitational treatment was normalized to the interval from fertilization (0.0) to first cleavage (1.0) and expressed as a fraction of this interval. The treatments were done at room temperature (from 19 to 24 °C) with cleavage times of 100 to 70 min, respectively. In the case of 2, 3 and 4, eggs in gelatin were inclined 90° with the sperm entry side uppermost, at 1g, by turning the dish on its side. For 5, 7, and 8, eggs in gelatin were centrifuged with the sperm entry side towards the centre of the rotor, that is, uppermost in the centrifugal field. In 6, eggs were centrifuged in this orientation for 4 min and then the dishes were kept inclined at 90° for 90 min with the sperm entry side uppermost. After the gravitational treatment, the eggs were returned to the upright position and incubated at 15 °C for development. After 48 h the embryos were scored for the position of the neural groove and neural folds. The position of the neural groove is recorded as the angle between two vectors originating at the vegetal pole, one passing along the neural groove and one passing through the sperm entry point. An angle of 180° indicates that the neural groove is opposite the side of sperm entry; an angle of 0° indicates that the groove is on the same side.

from 0.5 to 0.8 (refs 17–19). Therefore, we needed to test whether forces >1g could shift the dorsal-ventral axis at still later times.

We used centrifugation to achieve more rapid and extensive movement of the egg contents in the refractory period. Eggs were first embedded and oriented uniformly in a dish of molten gelatin, which, like Ficoll, dehydrates the perivitelline space, and on solidification also holds the eggs in a fixed position. The dish was placed in the centrifuge with the SEP side of the eggs towards the centre of the rotor, so that the centrifugal force would move materials away from it. This orientation is equivalent to that of an egg turned SEP upwards in the gravitational field. After centrifugation, the embedded eggs were incubated in the normal orientation and later scored for the position of definitive dorsal structures, that is, the neural groove and folds, relative to the position of the original SEP. Data are summarized in Table 1. Uncentrifuged control embryos, left in gelatin in the normal vertical orientation, formed the dorsal side at a position averaging 138° from the SEP, a value slightly lower than the 158° found for the blastopore lip position in controls of Fig. 2, perhaps due to limited movement of the gelatin-embedded embryos after gastrulation. As a further control, embedded eggs were turned through 90° at 1g for 20 min starting at 0.4—these developed dorsal structures at a position 20° from the SEP, again indicating the strong effect of gravity on pre-grey crescent eggs. By the time 0.9, eggs turned horizontally at 1g for 20 min, or even for 90 min, in gelatin were completely refractory to gravity.

However, if eggs were centrifuged at 26g for 4 min at 0.9, while oriented with the SEP side uppermost in the centrifugal field, there was a detectable effect on the prospective axis; the dorsal side of the embryo developed, on average, 112° from the SEP. If the 4-min centrifugation at 26g was followed by a 90-min period of horizontal inclination at 1g, the effect was greater, moving the dorsal side to within 74° of the SEP. Finally, if eggs at 0.95 were centrifuged at 50g for 4 min, the dorsal-ventral axis of the embryo was essentially reversed, the dorsal side of the embryo developing within 20° or 30° from the SEP side. The latter treatment was effective until first cleavage, when the developing furrow weakened the egg so that it did not survive the centrifugation. Thus, while the post-crescent period

is characterized by an increasing resistance to gravity, the prospective axis can still be shifted as completely as in the pre-crescent period, provided more force is applied. Thus, the locus for dorsal development can be completely dissociated from its normal topographical relationship to the grey crescent, well after the time of crescent formation.

Double axis formation

The importance of the grey crescent in dorsalization has been most directly demonstrated by the production of double-axis embryos from eggs grafted with pieces of grey crescent cortex⁸. However, our data contradict such a role for the grey crescent. It seemed unlikely that the different results could be reconciled by describing the grey crescent cortex as active on transplantation while at the same time easily rearranged or replaced in the egg when exposed to gravitational force. Alternatively, we must ask whether the transplantation results could in some way be an artefact of gravity and not an effect of cortical transplantation. While exploring the effect of increasing centrifugal force on axis position, we found that double-axis embryos can indeed arise at high frequency within a narrow range of conditions of centrifugation. As shown in Table 2 (groups 1 and 3), at a force of 30g applied for 4 min in the period 0.5–0.7, 60–70% of the eggs developed into neurulae with double, opposite, partial or complete sets of dorsal structures, essentially as Siamese twins. Double axes form only if the egg is centrifuged with the SEP side oriented towards the centre of the rotor, that is, in the position uppermost in the centrifugal field. As shown in Table 2 (group 2), when the SEP is directed away from the centre, no twins are formed, and the single dorsal side is centripetal, where the crescent had resided. In this orientation, gravity presumably just

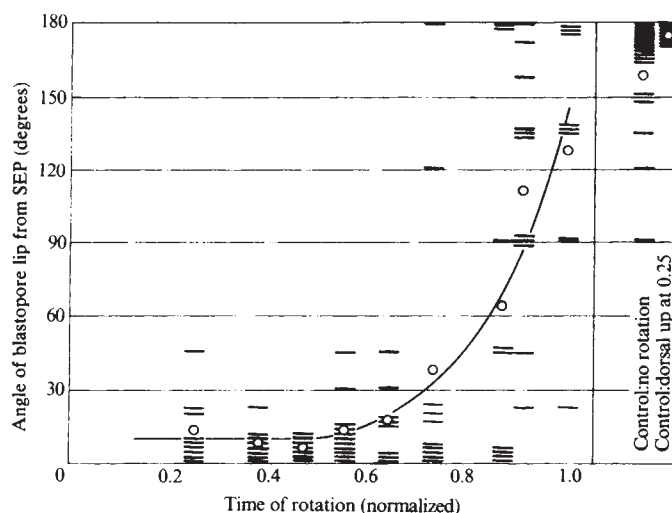


Fig. 2 Position of the dorsal blastopore lip in embryos from *X. laevis* eggs rotated at various times after fertilization. Eggs were fertilized and dejellied as described in Fig. 1 legend and placed in 20% MR containing 5% Ficoll. The eggs were marked at the prospective ventral equator, that is, on the side of the SEP as described in Fig. 1 legend, and were then left in a vertical position with the animal pole upwards, until the time of rotation when they were turned (90° rotation with SEP uppermost). Eggs remained in the rotated position for 60 min and were then returned to a vertical orientation and allowed to develop in 20% MR. The position of the first appearance of the blastopore lip, ~9–15 h after fertilization (16–21 °C), was scored as the angle between the lip and the dye mark, as described in Fig. 1 legend; 0° indicates superposition of the dye mark and lip, whereas an angle of separation of 180° indicates opposition of the mark and lip. The angle of separation is given on the ordinate, and the time at which the egg was rotated is shown on the abscissa. The time is normalized, with fertilization set as 0.0 and the start of first cleavage set at 1.0. This period ranges from 70 to 130 min depending on the temperature. Data for individual eggs at each time are given as horizontal dashes, while the average for that time is given as an open circle. Control results are shown at the right hand side of the figure: eggs were either left without rotation, or were turned with the dye mark (SEP side) downwards. Note that the latter controls gave an even sharper population response than unrotated controls, for the average angle of separation of the lip and mark. Note also in the experimental series the heterogeneity of the response in the time range 0.7–0.9, when some eggs were affected totally by gravity, a few were not affected at all, and others gave intermediate angles of separation.

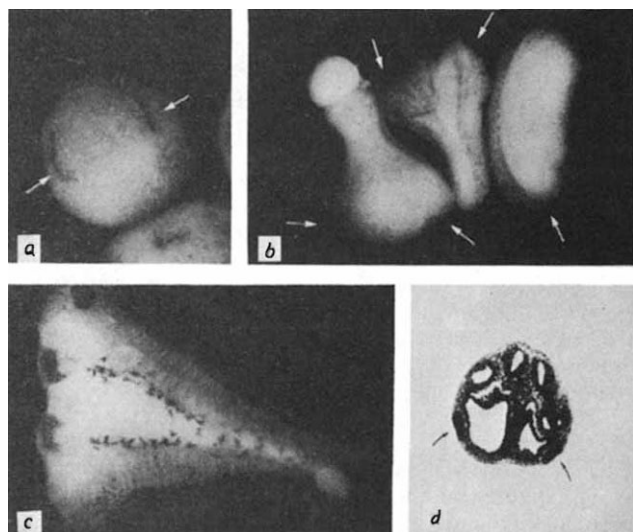


Fig. 3 Twin embryos produced from eggs removed from the fertilization envelope and turned ventral side uppermost, before first cleavage. *a*, Early gastrula with two blastopores, indicated by arrows; *b*, three late neurulae, two with double neural grooves and one a control embryo with a single neural groove. The anterior end of each groove is indicated by an arrow. *c*, Siamese twin at hatching, at about stage 38. One of the axes carries a less well developed head, lacking eyes, although the sucker is present and the trunk is complete. *d*, Section of a twin embryo, at the posterior head level. Two neural tubes are visible at the top, close together, and two mouth cavities are present at the bottom. Arrows indicate two suckers. The axes were more separated in the mid-trunk region. Paraffin sections were 7 μ m thick and were stained with nuclear fast red-aniline blue-orange G.

reinforces the effects of the SEP and the grey crescent on axis determination. Furthermore, even in the orientation favourable for twinning, the extent of rearrangement is critical: at still higher centrifugal force, twins do not form and the single axis forms on the SEP side, that is, that controlled by centrifugal force. This is shown in Table 2, group 4; eggs were centrifuged with the SEP towards the centre of the rotor, as is suitable for twinning, but were subsequently inclined horizontally with the side of the SEP uppermost, to continue lateral displacements of the egg contents at 1g for 90 min. In this case, no twins were formed and the single axis derived from the SEP side, not the side of the grey crescent. Thus, twins seem to develop when the gravitational force is strong enough to create a new dorsal localization in the egg but not so strong as to eliminate the old (SEP-grey crescent-directed) one.

Reservations about cortical grafting results

As high centrifugal force produces double-axis embryos, we attempted to duplicate the conditions at 1g used by Curtis⁸ in his cortical transplantation experiments, to determine whether gravity could also act as a twinning agent there. When the egg was removed from the fertilization envelope by manual dissection and allowed to settle on to an agar surface with its SEP side uppermost, twins were in fact produced. The frequency was quite variable from one egg batch to the next and as shown in Table 2, we found two batches (that is, from two different frogs) giving 50–70% double-axis embryos, three batches giving 30–49%, eight giving 10–29% and eight giving 0–9%. Figure 3*a* shows an example of a double blastopore of an early gastrula from an egg turned horizontally with the SEP side uppermost starting at 0.8, and left in this orientation until the 16-cell stage. Examples of double neurulae and of a hatching-stage Siamese twin are shown in Fig. 3*b* and *c*, respectively. Histological analysis confirmed the presence of doubled internal dorsal structures (Fig. 3*d*).

With regard to the conditions required for twinning by this procedure, it should first be mentioned that eggs removed from the fertilization envelope lack a perivitelline space and remain in the orientation in which they are put intentionally or by chance.

Therefore, just as in the Ficoll treatment, they cannot return to the vertical orientation and are susceptible to gravity-induced displacements of the egg contents. Second, twinning has been observed by us only in eggs oriented with the SEP side, that is, the prospective ventral side, uppermost. Table 2 records no double-axis embryos for two sets of eggs oriented with the SEP directed downwards, that is, prospective dorsal side uppermost; eggs of these same batches gave 20% and 35% twins when oriented SEP upwards. This requirement is the same as found for twinning by centrifugation and implies the importance of having gravity oppose rather than reinforce the effects of the sperm and grey crescent on axis selection. Third, extreme flattening of the egg which occurs on its removal from the fertilization envelope may also be an important precondition for twinning at 1g, thereby explaining why we rarely observed twins (never more than 5%) in our experiments when eggs were rotated SEP side uppermost in Ficoll or gelatin, as these eggs largely retain their spherical shape. Thus, with the proper choice of conditions, gravity can cause the formation of double-axis embryos in *X. laevis*. Double blastopores and secondary embryonic axes due to gravitational effects have been previously reported by Pasteels¹ using an axolotl egg turned with the prospective ventral side directed upwards shortly after grey crescent formation, by Kubota²⁰ with *Rana nigromaculata* eggs inclined horizontally at the time of crescent formation, and by Schultz²¹, Penners and Schleip²² and Penners²³ with inverted and flattened *R. fusca* eggs.

Because of these results, we question Curtis' interpretation⁸ that double-axis embryos in his experiments owe their origin to the implantation of grey crescent cortex. To implant the graft, Curtis had to remove the fertilization envelope of the recipient egg, which means that the egg was no longer free to control its

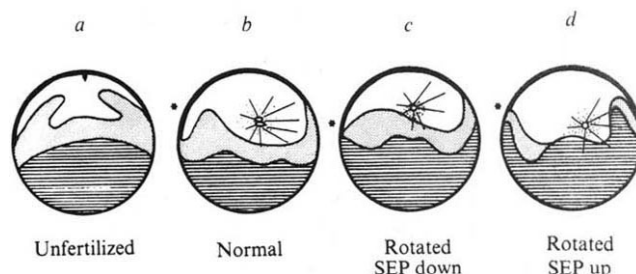


Fig. 4 Dorsal-ventral rearrangements of the cytoplasmic contents of *X. laevis* eggs. Eggs were fixed at various times in Bouin d'Hollande fixative, dehydrated, embedded in paraffin, sectioned at 6 μ m along the prospective dorsal-ventral axis and stained with azofuchsin-aniline blue-orange G. Median sections along the prospective dorsal-ventral axis were examined for regional differences in the size and number of yolk platelets, the presence of pigment granules, aster and spindle fibres, and pronuclei. Schemes of representative sections are shown; this was necessary as only the highest-quality colour reproductions preserve the regional differences seen under the microscope. Photographs of actual sections will be presented elsewhere¹⁴. In the drawings, the region of large, closely packed vegetal platelets is indicated by cross-hatching; the intermediate size, closely packed equatorial platelets are indicated by dots, and the animal hemisphere region of small dispersed platelets and abundant cytoplasm is indicated by an open area. In all cases the egg is oriented animal hemisphere uppermost, and in the case of fertilized eggs, the SEP is at the left side of the egg, at or slightly above the equatorial level, as indicated by the asterisk. *a*, Drawing of an unfertilized egg, showing the radial distribution of contents. At the animal pole of the egg is the second meiotic spindle. *b*, A fertilized egg fixed at 0.6, well after the grey crescent became visible. The egg has been kept in the normal vertical orientation. Note the upward trail of intermediate and large yolk platelets on the prospective dorsal side of the egg. The region of the grey crescent is indicated by the raised boundary of pigment granules in the cortex. Dorsal structures will arise from this region of the egg, that is, opposite the SEP side. The sperm aster with paired pronuclei is still clearly visible. *c*, An egg turned horizontally with the SEP down from 0.26–0.5, fixed at 0.6. Note the trail of large and intermediate yolk on the right-hand side, occupying about the same position as in a normal (non-rotated) egg. This egg, like a non-rotated egg, would develop dorsal structures from the right side, the side opposite the SEP (asterisk). *d*, An egg which was turned horizontally with the SEP side upwards, starting at 0.28 and remaining until 0.5. It was subsequently fixed at 0.7. Note the trail of yolk at the left-hand side of the egg, the SEP side (asterisk). The horizontal orientation allowed gravity to displace cytoplasmic contents away from the SEP side, leaving the trail of yolk behind. The dorsal structures of the embryo will arise from the SEP side of this egg, instead of opposite the SEP.

Table 2 Secondary axis formation (twinning) by *X. laevis* eggs perturbed by centrifugal force or gravity

Starting time	Duration (min)	Force (g)	Angle of inclination of egg	No. of eggs	% Embryos with double axes
(1) 0.65	4	30	90°, SEP in	20	70
(2) 0.65	4	30	90°, SEP out	13 (1 frog)	0
(3) 0.60	4	30	90°, SEP in	14 (1 frog)	64
(4) 0.60	4 + 90	30 + 1	90°, SEP in/up	15	0
(5) } (6) } (7) } (8) }	0.5–1.0	Until 16-cell stage	1	45–90°, SEP up	149 (8 frogs) 0–9 159 (8 frogs) 10–29 50 (3 frogs) 30–49 26 (2 frogs) 50–70
(9) 0.5–1.0	Until 16-cell stage	1	45–90°, SEP down	34 (2 frogs)	0

Eggs were fertilized, dejellied, and either embedded in 9% gelatin/30% modified Ringer's solution (MR)^{28,29} with the SEP towards the marked side of the dish or immersed in 6% Ficoll–20% MR²⁸. The gelatin-embedded eggs (rows 1–4) were centrifuged as described in Table 1 legend at 700 r.p.m. (30g) for 4 min, starting at 0.60 or 0.65 as indicated. During centrifugation, the eggs of rows 1, 3 and 4 were oriented with the SEP side towards (SEP in) the centre of the rotor whereas the eggs of row 2 had the SEP side away (SEP out) from the rotor centre. These orientations corresponded to having the animal–vegetal axis of the egg horizontal (90°) in the centrifugal field with the SEP side up or down, respectively. In the case of Ficoll-treated eggs (rows 5–9), the fertilization envelopes were removed manually using forceps, and starting in the time period 0.5–1.0 the eggs were turned SEP side uppermost (rows 5, 6, 7 and 8) or down (row 9) on an agar surface in 20% MR containing 50 µg ml⁻¹ gentamycin. Eggs were kept in this orientation until the 16- or 32-cell stage and then were transferred to hemispheric wells in agar in 20% MR–gentamycin to enhance advanced development of the embryos. The treatments were done at room temperature (19–23 °C). The number and completeness of the axes were scored at stages from late neurula to advanced tailbud when suckers were identifiable.

orientation. As shown in ref. 8, Fig. A, B, his recipient eggs were inclined 45°–90° to the side in the period of grafting or recovery, which is the orientation required for gravity to cause lateral displacements of the egg contents. We assume that eggs receiving grafts in the ventral margin were turned slightly with this side uppermost, to receive the graft; this would be the condition for twinning by gravity. Implantations in this position are the ones for which Curtis observed double-axis embryos and which led him to conclude the dorsalizing activity of the graft. The critical control for these experimental manipulations would have been a grafting of a piece of non-grey crescent cortex (for example, ventral margin cortex) into the ventral side of a recipient egg. If the source of the cortex is critical, such recipients should develop only a single axis; if unrecognized gravitational effects are responsible, the recipients should still twin. Although Curtis did not report this control, he did use several others involving grafts of grey crescent, animal pole and ventral cortex, but all were implanted in the dorsal (that is, grey crescent) side of the egg. If these eggs were turned slightly with dorsal side uppermost to receive the graft, they would not be expected to give twins, according to our results, as, in this orientation, gravity would only reinforce the normal sperm and grey crescent-dependent dorsalization of the egg. In fact, in Curtis' experiments, all implantations to the dorsal margin gave single-axis embryos. We suggest that these were not appropriate controls for the ventral implantations, all of which gave twins.

Although we cannot prove that Curtis' grafts of grey crescent cortex did not have transplantable dorsalizing activity, we question such an interpretation since we can obtain twinning without cortical transplantation, simply by exposing eggs to experimental conditions close to, if not identical, to those used by him. It seems plausible to us that the critical agent was not the cortical graft but gravity acting on an egg that was turned ventral side uppermost.

The role of the grey crescent

We suggest that the grey crescent is a manifestation of an early dorsalizing process but is not itself a transplantable dorsal determinant or source of a lasting cortical field. Although the mechanism of grey crescent formation is unknown, and may differ among various amphibia²⁴, Lovtrup²⁵ and Palaček *et al.*¹³ suggest that in *X. laevis* the crescent develops opposite the sperm entry side because a large asymmetric contraction of the egg cortex draws pigment granules of the animal hemisphere towards the sperm entry side, leaving a visibly depigmented crescent on the dorsal side. We suggest that this asymmetric contraction as a whole is an early dorsalizing mechanism important for establishing regional cytoplasmic differences along the prospective dorsal–ventral axis of the egg. The target of the contraction would not be the cortex, but deeper, movable cytoplasmic materials. Pasteels' observed in sections of post-grey crescent eggs of *R. fusca* a trail of displaced vegetal yolk

associated with the cortex on the dorsal side and suggested that it was pulled upwards during grey crescent formation. Klag and Ubbels²⁶ observed similar dorsal displacements in eggs of *Discoglossus pictus*.

Figure 4a, b shows schematic diagrams of actual sections of *X. laevis* eggs, in one case before fertilization when the egg contents are arranged with radial symmetry and in the other case at 0.6 when the egg has already acquired a detectable asymmetry along its dorsal–ventral axis. The displacement of yolk is discernible on the prospective dorsal side, while the ventral side has a quite different distribution of materials. Actin filaments of the cortex presumably participate in the contraction, but microtubules must also play a part since inhibitors such as colchicine and vinblastine block grey crescent formation and cytoplasmic displacements^{14,18}. We propose that a vectorial contraction of the cortex normally induces a rearrangement of the egg's cytoplasmic contents, forming a cytoplasmic localization necessary for the eventual induction of dorsal mesoderm.

Although in *X. laevis* the cortical contraction probably operates only in the time period 0.5–0.8, gravity may be able to generate a comparable dorsal–ventral asymmetry at earlier or later times, perhaps until the egg cytoskeleton or cleavage furrows interfere. Figure 4c shows a schematic section of an *X. laevis* egg rotated horizontally with SEP directed downwards, an orientation in which gravity reinforces normal axis formation. The cytoplasmic distribution of this egg is equivalent to that of the normal egg shown in Fig. 4b; in Fig. 4d a similar egg has been turned horizontally with the SEP uppermost, an orientation leading to axis reversal. In this case the trail of vegetal yolk appears on the same side as the SEP, roughly the mirror image of the normal egg in Fig. 4b. Thus, the cytoplasmic distributions correlate with axis reversal. We have not yet sectioned eggs obtained in conditions which favour twinning, but we might expect double symmetrical trails of yolk. Cytological studies of normal, rotated and centrifuged eggs will be presented in detail elsewhere¹⁴.

Initially the sperm must in some way determine the position of the prospective dorsal–ventral axis of the egg, because the grey crescent, blastopore, and dorsal structures normally arise on the side opposite its random point of entry. Since the cortex contracts towards the side of sperm entry, the question becomes one of how the sperm orients this contraction. Soon after entering the egg, the sperm produces a large aster, organized presumably by its centriole^{18,27}. The astral microtubules cause a slight redistribution of cytoplasmic contents along the future dorsal–ventral axis. The antimicrotubule drugs colchicine and vinblastine block aster assembly and in their presence the cytoplasmic displacements do not occur¹⁴. Similarly, artificially activated eggs of *X. laevis*, lacking a functional centriole, do not have these early displacements. We suggest that this initial slight aster-driven asymmetry cues the direction of the cortical contraction, that in turn leads to the major cytoplasmic

redistribution shown in Fig. 4b. It seems consistent that the *X. laevis* egg is more sensitive to the axis-orienting effects of gravity in the pre-crescent period than in the post-crescent period, since at early stages, gravity, like the sperm aster, needs only to create a small lateral displacement of cytoplasmic materials to cue the cortical contraction. In the post-grey crescent period, larger forces, afforded by centrifugation, are required to reverse the cytoplasmic distribution accomplished by the contraction and to create a new and opposite one.

In summary, the important dorsal-ventral regional differences arising in the fertilized egg before first cleavage appear to reside not in the grey crescent cortex but in cytoplasmic materials reorganized normally by the sperm aster and cortical contraction, or experimentally by gravitational force.

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1. Pasteels, J. J. *Archs Biol., Paris* **49**, 629–667 (1938); *Folia biotheor.* **3**, 83–108 (1948); *Adv. Morphogen.* **3**, 363–388 (1964).
2. Brachet, J. *Curr. Topics dev. Biol.* **11**, 133–186 (1977).
3. Nieuwkoop, P. D. *Wilhelm Roux's Arch. dev. Biol.* **162**, 341–373 (1969); **163**, 298–315 (1969); *Adv. Morphogen.* **10**, 1–39 (1973); *Curr. Topics dev. Biol.* **11**, 115–132 (1977).
4. Gerhart, J. C. in *Biological Regulation and Development* Vol. 2 (ed. Goldberger, R.) 133–316 (Plenum, New York, 1980).
5. Spemann, H. *Embryonic Development and Induction* (Yale University Press, New Haven, 1938).
6. Spemann, H. & Mangold, H. *Wilhelm Roux's Arch. dev. Biol.* **100**, 599–638 (1924).
7. Dalcq, A. & Pasteels, J. *Archs Biol., Paris* **48**, 669–710 (1937).
8. Curtis, A. S. G. *J. Embryol. exp. Morph.* **8**, 163–173 (1960); **10**, 410–422 (1962).
9. Nieuwkoop, P. D. & Ubbels, G. A. *Wilhelm Roux's Arch. dev. Biol.* **169**, 185–199 (1972).
10. Boterenbrood, E. C. & Nieuwkoop, P. D. *Wilhelm Roux's Arch. dev. Biol.* **173**, 319–332 (1973).
11. Nieuwkoop, P. D. & Florschütz, P. *Archs Biol., Paris* **61**, 113–150 (1950).
12. Keller, R. E. *Dev. Biol.* **51**, 118–137 (1976).
13. Palaček, J., Ubbels, G. A. & Rzehak, K. *J. Embryol. exp. Morph.* **45**, 203–214 (1978).

While the cortex, including the grey crescent, would be part of the cellular machinery used by the egg to generate a dorsal cytoplasmic localization, it would not itself be a lasting repository of developmental information. We do not know which important materials undergo redistribution on the dorsal side of the egg, nor is it known how these materials serve in the formation of the inductively active vegetal cells of the mid-blastula.

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14. Ubbels, G. A., Hara, K., Korster, C. H. & Kirschner, M. W. (in preparation).
15. Ance, P. & Vintemberger, P. *Bull. biol. Fr. Belg.* **31**, 1–182 (1948); *Archs Anat. microsc. Morph. exp.* **38**, 167–183 (1949).
16. Kirschner, M. & Hara, K. *Mikroskopie* **36**, 12–15 (1980).
17. Ance, P. & Calame, S. C. *r. hebdom. Séanc. Acad. Sci., Paris* **248**, 893–895 (1959).
18. Manes, M. E., Elinson, R. P. & Barbieri, F. D. *Wilhelm Roux's Arch. dev. Biol.* **185**, 99–104 (1978).
19. Manes, M. & Elinson, R. P. *Wilhelm Roux's Arch. dev. Biol.* **189**, 73–76 (1980).
20. Kubota, T. *J. Embryol. exp. Morph.* **17**, 331–340 (1967).
21. Schultze, O. *Wilhelm Roux's Arch. dev. Biol.* **1**, 160–204 (1894).
22. Penners, A. & Schleip, W. *Z. wiss. Zool.* **130**, 305–454 (1928); **131**, 1–156 (1928).
23. Penners, A. *Wilhelm Roux's Arch. dev. Biol.* **116**, 53–103 (1929); *Z. wiss. Zool.* **148**, 189–220 (1936).
24. Elinson, R. P. *Symp. Soc. dev. Biol.* **38**, 217–234 (1980).
25. Lovtrup, S. *Wilhelm Roux's Arch. dev. Biol.* **156**, 204–248 (1965).
26. Klag, J. J. & Ubbels, G. A. *Differentiation* **3**, 15–20 (1975).
27. Subtelny, S. & Bradt, C. *J. Morph.* **112**, 45–60 (1963).
28. Kirschner, M., Gerhart, J. C., Hara, K. & Ubbels, G. A. *Symp. Soc. dev. Biol.* **38**, 187–215 (1980).
29. Scharf, S. R. & Gerhart, J. C. *Dev. Biol.* **79**, 181–198 (1980).

LETTERS TO NATURE

Estimates of total quantity of meteorites in the East Antarctic ice cap

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Since 1969 ~5,000 meteorite fragments have been recovered from two regions, the Yamato Mountains and Victorialand, on opposite sides of the East Antarctic ice cap^{1,2}. Based on a steady-state model for the ice cap³, and current estimates of meteorite influx⁴, a model is developed here which predicts that the steady-state number of meteorites being carried in and on the ice is at least 760,000. Most of these are being carried within the ice and are only exposed at peripheral regions by a combination of wind ablation and blockage of ice movement by protruding mountain barriers. The large steady-state population of meteorites does not require unusual conditions of influx. It is solely the cold, dry climate which preserves virtually all meteorites that fall except for the fragile, porous carbonaceous chondrites. The same model applied to the Greenland ice cap indicates a steady-state population of ~61,000 meteorites.

The East Antarctic ice cap has several properties that permit some simplifications in developing a model for it, without loss of generality. The area is roughly elliptical with major and minor diameters of ~4,000 km and 3,000 km, respectively. It has an area of 9.4×10^6 km² and a single major centre of ice flow. The calculated circumference is 1.11×10^4 km. For the purpose of this model, the calculation is simplified if an equivalent circle is used with the same area and almost identical circumference. The equivalent circular continent would have a radius of 1,730 km (area 9.4×10^6 km², circumference = 1.09×10^4 km).

Meteorites falling randomly over the ice cap are carried in and on the ice radially coastwards. Initially, we consider that there is

no blockage of movement and each meteorite reaches the coast where it is lost by calving of the ice into the sea. After some time a steady-state population, Q , of meteorites will be reached in the ice cap. A simple relationship can be derived, $Q = sF/2v$, where F is the influx rate (meteorites yr⁻¹ for the total area, 9.4×10^6 km²), s is the distance travelled (m), and v is the mean velocity of travel (m yr⁻¹). The time for the steady state to be established is T (yr) = s/v . Based on a steady-state ice cap profile, as well as actual measurements in the field, the velocity near the coast is higher than in the interior.

Nagata² has developed a model for the longitudinal profile of a steady-state ice cap. In it he derives analytical expressions relating v to s and a parameter, b , the accumulation rate of ice expressed in m yr⁻¹ of water equivalent.

To determine how v varies with distance from the centre to the edge some estimates of b are needed. For East Antarctica Paterson⁵ states that accumulation ranges from $b = 0.03$ m yr⁻¹ in the central part to ~0.60 m yr⁻¹ near the coast. Measured velocities range from ≤ 1 m yr⁻¹ in the central part to over 300 m yr⁻¹ near the coast^{5,6}. These observations were used in conjunction with the Nagata model to model the velocity from the centre to the coast. A tabulation of velocities was prepared but no attempt was made to fit them by an analytical expression.

Hughes⁴ has summarized data on meteorite influx rates. The value of F to be used in the calculation of Q depends on the mass of the smallest meteorite to be considered. If dust grains are considered then Q would be very large; if ton-size meteorites are considered then Q would be very small. In Antarctica, within an area of concentration, specimens as small as 0.1–0.87 g have been collected^{7,8}. These, however, are clearly fragments from larger masses that have suffered extensive fragmentation. Thus, an estimate of the fragmentation factor is needed.

Based on the proportion of irons and pallasites to stone meteorites in the Yamato recovery region, relative to worldwide observed falls, Nagata² estimates a factor of 3. The same analysis applied to the Victorialand finds⁸ gives a value of 5. King *et al.*⁹ have attempted to pair many of the Victorialand specimens

based on field occurrence, macroscopic and microscopic appearances. Their suggested pairings indicate a factor between 3 and 4. Thus, a factor of 4 ± 1 seems to be indicated, which seems remarkably low. Antarctic meteorites suffer four fragmentation processes: (1) normal break-up during atmospheric passage (Hellyer¹⁰ states that about half of observed falls are multiply fragmented); (2) fragmentation on impact on ice; (3) crushing while being carried within the ice during transport; (4) frost-breakage while exposed in concentration areas near the ice periphery (dark rocks are warmed above the freezing point of water during summer months, allowing moisture within them to melt and then refreeze during the dark winter months). A better method to determine fragmentation would be to compare the distribution of recovered masses with the masses of observed meteorite falls. The data on the recoveries in Victorialand through 1979 have been tabulated by Score *et al.*⁸. The distribution of masses is highly skewed. The mean mass is 1,910 g, while the median is 235 g and the mode is 15 g. Hughes¹¹ has plotted the distribution of recovered masses among 755 observed falls. The histogram shows a mode at 3 kg. If we assume that the mode of observed falls is a good measure of the highest frequency of meteorite masses that survive entry, then comparison of the mode of the Victorialand finds, 15 g, with this 3 kg figure indicates the most frequent fragmentation factor is 200. This seems to be a more reasonable number. Although iron meteorites clearly fragment less readily than stone meteorites, data on observed falls indicate that over 91% are stones. Thus, based on data for observed falls, the most frequent mass surviving entry is 3 kg. In Antarctica the most frequent size of fragment is 15 g. A 15-g meteorite is readily observed on the ice in Antarctic field conditions.

Hughes⁴ has fitted an equation to the observed data on recovered masses (non-Antarctic) for the mass range ≥ 16 kg. Extrapolation of this equation to 3 kg gives a worldwide influx rate of 3,500 meteorites yr^{-1} . This number is in good agreement with an estimate of 3,300 based on Hughes' analysis of data presented by Brown¹² and tabulated in ref. 4. Both Halliday and Hughes (personal communications) agree this is the most reasonable figure to use based on current data. In addition, Halliday¹³ has determined a latitude effect by analysis of meteorite orbits. This effect depends on the initial velocity of a meteorite encountering the Earth. Those with a velocity of 15 km s^{-1} will be $\sim 20\%$ less frequent at high latitudes (70° – 90° , N or S) relative to equatorial latitudes; those with a velocity of 25 km s^{-1} will be 40–50% less frequent at high latitudes. ReVelle¹⁴ has developed a model for the mass-loss (ablation) of chondrites falling through the Earth's atmosphere. From it one can conclude that most meteorites that survive entry are the slower ones⁴. Thus, for Antarctica a reduction of 20% will be used for the latitude effect. The value of F for East Antarctica is then 48.6 meteorites of 3 kg mass or larger per year.

Tabulated mean values of v were used in each of 10 concentric subregions, of equal area, from the centre to the edge of the continent, and the respective values of Q were determined and summed. The steady-state quantity of meteorites is $Q = 529,000$ (1.06×10^8 fragments). The time required for the steady state to be established is 22,000 yr, which is short compared with the age of the ice cap¹⁵. In these computations it was assumed that effects due to ice surging movement average out over this time span.

These computations assume that no blockage or retardation of ice occurs. In reality $\sim 47\%$ of the East Antarctic perimeter consists of protruding mountains that cause significant retardation of ice movement. In the vicinity of the Yamato Mountains horizontal velocities of 1 to 0.5 m yr^{-1} have been measured². Assuming 1 m yr^{-1} in the vicinities of these barriers, and 47% of the perimeter affected, the effect is to increase the value of Q to 760,000 meteorites (1.5×10^8 fragments).

The computations, so far, have assumed that each meteorite lands on the ice and is carried, either in it or on it, in a straight line path to the perimeter. Clearly, this cannot be the case as ice-buried topographic highs, subsidiary flow centres on the ice

(such as the Fuji Divide near, and upstream from the Yamato Mountains), and local variations in accumulation rate cause the ice to move along on less-than-direct lines. For any path that meanders from the direct path the effect is to increase the value of Q . There is no way, quantitatively, to assess the magnitude of this effect over the whole ice cap. Thus, the value of Q determined above is a minimum.

Finally, one additional process can affect the value of Q . It has been assumed so far that v is not a function of depth within the ice at which a meteorite is carried. Clearly, as accumulation takes place over time, any meteorite that fell in an earlier time will be buried as it is carried along. Variation of velocity with depth is not believed to be a large factor. Velocity is considered almost constant from the ice surface down to about half of full depth¹⁶. What happens below half depth, especially near the bottom, is not thoroughly understood and several possibilities exist¹⁷. In terms of Nagata's model this factor does not seem to affect Q significantly.

Considering all the assumptions and simplifications in the foregoing model it can be concluded that the East Antarctic ice cap contains, at any given time, the order of 760,000 individual meteorites, in the mass range ≥ 3 kg, and comprising $\sim 1.5 \times 10^8$ fragments. If these numbers are applied to the Yamato Mountains, and if the velocity near the Yamato barrier ($\sim 40 \text{ km wide}$) is taken to be 1 m yr^{-1} , the ice field in front of the mountains would have a steady-state population of about 3,000 individual meteorites. This does not take into consideration the lateral convergence effect described by Nagata², which would increase this number. Even at that, this estimate is of the order of magnitude estimated by Yanai¹⁸ of $< 8,000$ for this region.

The mechanism by which unusual concentration fields occur, as proposed by Nagata², has permitted large numbers of meteorite fragments to be recovered within relatively small areas. On the other hand, the work of Cassidy and colleagues in southern Victorialand^{1,19} shows that concentration fields are not the only way Antarctic meteorites will be recovered. Several meteorites have been encountered that were clearly moving along alone. A prime example of this is the Mt Baldr meteorite (two main fragments with missing fragments) that was found on the surface of a segment of ice cap within a few kilometres of, and moving towards, a major ice-fall onto Wright Upper Glacier at the head of one of the Dry Valleys; no barrier was present. While lateral concentration helps in recovering large numbers of specimens, the key factor in Antarctica is the low rate of weathering at low temperatures, which preserves most of the meteorites that land on the ice cap. This is borne out by the terrestrial ages of Antarctic meteorites which are, on the average, significantly older than finds from lower latitudes²⁰. Because of the general condition of ablation by wind in peripheral regions of the ice cap (an integral part of the Nagata steady-state profile model³) meteorites carried within the ice have a high probability of being exposed in these regions. This means that bare blue ice, free from snow cover in peripheral regions, can yield meteorites with or without lateral concentration. Thus far, all meteorites found in East Antarctica, including the first finds in earlier decades of this century, have been recovered from peripheral regions.

Antarctica, however, cannot be considered a place where every meteorite is preserved. Because of the great detail in which Japanese field teams have studied the Yamato Mountains concentration area, data on the distribution of meteorite types there are quite good. Note that carbonaceous chondrites are under-represented, relative to worldwide recovery ratios, by a factor of three². This indicates that their low mechanical strength, plus their high porosity causes them to be preferentially destroyed by a combination of crushing and weathering when carried within the ice.

If this model is applied to the Greenland ice cap (without consideration of any coastal barriers) it suggests that $\sim 61,000$ meteorites (1.2×10^7 fragments) are in steady-state residence. Unfortunately, most of the Greenland ice cap is snow covered. Meteorological conditions are different from those in Antarctica

because of its subpolar position. Only a couple of relatively snow-free regions in north-east Greenland seem suitable for potential meteorite recoveries.

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1. Cassidy, W. A., Olsen, E. & Yanai, K. *Science* **198**, 727-731 (1977).
2. Nagata, T. *Proc. 2nd Symp. Yamato Meteorites* 70-92 (1978).
3. Nagata, T. *Antarctic Rec.* No. 60, 13-27 (1977).
4. Hughes, D. W. in *Solid Particles in the Solar System* (eds Halliday, I. & McIntosh, B. A.) 207-210 (International Astrophysical Union, 1980).
5. Paterson, W. S. B. in *Dynamics of Snow and Ice Masses* (ed. Colbeck, S.C.) 1-78 (Academic, New York, 1980).
6. Gow, A. J. in *Antarctica* (ed. Hatherton, T.) 221-258 (Methuen, London, 1965).
7. Yanai, K. *Catalog of Yamato Meteorites*, 1-188 (National Institute of Polar Research, Tokyo, 1979).
8. Score, R. et al. *Antarctic Met. Newslett.* **4**, 1-144 (1981).
9. King, T. V. V., Score, R., Gabel, E. M. & Mason, B. *Smithsonian Contr. Earth Sci.* No. 23, 12-44 (1980).
10. Hellyer, B. *Earth planet. Sci. Lett.* **7**, 148-150 (1969).
11. Hughes, D. W. *Meteoritics* (submitted).
12. Brown, H. J. *geophys. Res.* **66**, 1316-17 (1961).
13. Halliday, I. *Meteoritics* **2**, 271-278 (1964).
14. ReVelle, D. O. *Nat. Res. Council Canada, Planet. Sci.* SR-76-1 (1976).
15. Shackleton, J. J. & Kennett, J. P. *Init. Rep. DSDP Leg 29* (1965).
16. Raymond, C. F. in *Dynamics of Snow and Ice Masses* (ed. Colbeck, S. C.) 80-114 (Academic, New York, 1980).
17. *Proc. int. glaciol. Soc.* (1979).
18. Yanai, K. *Mem. Nat. Inst. Polar Res. Spec. Iss.* **8**, 1-37 (1978).
19. Cassidy, W. A. *Smithsonian Contr. Earth Sci.* No. 23, 3-7 (1980).
20. Evans, J. C. & Rancitelli, L. A. *Smithsonian Contr. Earth Sci.* No. 23, 45-46 (1980).

Measurements of diamond lattice displacement by platelet defects with electron microscopic moiré patterns

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Among the diverse lattice defects occurring in natural diamonds, none has engendered so much controversy as the 'platelets', those planar defects which lie on {100} planes of the diamond matrix and whose diameters commonly lie in the range 10-100 nm. For many years, the only evidence for the existence of platelets was indirect, residing in the reported anomalous 'spike' diffuse X-ray reflections¹, but it was eventually accepted that these diffuse reflections could not be due to thermal vibrations but arose from static lattice disorder². Guinier³ and Frank⁴ independently pointed out that the defects responsible for the 'spike' reflections must be plate-like, lying in {100}. Direct proof of the existence of platelets came with the transmission electron microscope (TEM) observations of Evans and Phaal⁵, and it has been shown that the platelet structure is extrinsic, that is, that it forces apart the diamond matrix on either side of the platelet⁶. We now report a new and more reliable way of measuring the magnitude of matrix displacement produced by platelets.

Moiré techniques are widely used for measuring small displacements of one periodic object relative to another of similar periodicity. If the two periodic objects are crystal plates superposed on each other in nearly parallel orientation and simultaneously diffracting from the same Bragg planes, then relative displacement of the two plates can be measured to a small fraction of a nanometre, whether electrons or X rays are being diffracted⁷⁻⁹. When studying atomic-scale displacements due to lattice defects, interpretation can be straightforward if a defect-free comparison crystal (1) is superimposed on the defect-containing specimen crystal (2). In the case of dislocations in (2) outcropping at the interface between (1) and (2), the number of moiré fringes terminating at a dislocation outcrop is $\mathbf{g} \cdot \mathbf{b}$, where $\mathbf{g}$ is the reciprocal lattice vector of the active Bragg reflection and $\mathbf{b}$ is the Burgers vector of the dislocation^{8,9}. Furthermore, if crystal (2) contains a planar defect such as a stacking fault which involves a relative translation, $\mathbf{f}$, between crystal matrices on either side of the defect, and the defect outcrops at the interface between (1) and (2), then the moiré fringe pattern will appear faulted at the defect outcrop, the fringe displacement at the outcrop being $\mathbf{g} \cdot \mathbf{f}$ fringe periods.

In our TEM studies of platelet defects in diamonds we prepared thin specimens parallel to (110). We then viewed platelets parallel to (001) edge-on and those parallel to (100) and (010) obliquely, when the electron beam is normal to the specimen surface (Fig. 5 of ref. 10 explains this geometry). The specimens are produced by sawing, mechanically polishing and ion-beam thinning. In one such specimen, cracking had occurred on the cleavage planes ($\bar{1}11$) and ($\bar{1}\bar{1}\bar{1}$) normal to the specimen plate, and on one of the cleavage planes inclined at 35.25° to its surface. Following cracking, small overthrusts had taken place, producing narrow strips of overlap in which the specimen laminae above and below were slightly rotated with respect to each other. The moiré patterns appearing in the overlap regions were usually complicated due to warping of the overlapping laminae and the mutual proximity of the platelets. However, one strip of overlap in which the moiré pattern can be straightforwardly interpreted is shown in Figs 1 and 2. This region was first observed in the high-resolution JEOL 200CX microscope at Oxford, operating at 200 kV. The set of two-beam diffraction contrast patterns reproduced here were photographed in Bristol with a Philips 400 microscope operating at 120 kV. (The corresponding electron diffraction patterns were also recorded.) The important reflections 004 and $\bar{2}20$ were photographed by dark-field (DF) as well as by bright-field (BF) technique. In the area shown, each lamina is ~33 nm thick. The vertically oriented boundaries of the overlap strip are the cleavage planes ($\bar{1}11$). The boundary sloping up towards the right at the top of Figs 1 and 2 is parallel to ($\bar{1}\bar{1}\bar{1}$). Except where the platelet strain fields influence the pattern, the moiré fringes are almost parallel and equispaced, and they lie parallel to the $\mathbf{g}$ vector of the Bragg reflection active. The basic pattern is thus a pure rotation moiré, the rotation measured from it being ~7 mrad.

The field in the Figs 1 and 2 contains nine platelets, four of which lie parallel to (001) and are seen edge-on giving sharp traces rotated 35° clockwise from the horizontal. The platelets in this specimen (as in many other specimens) are elongated along one of the $\langle 110 \rangle$ directions in the platelet plane; they tend to be lath-like, with their long pair of edges lying straight and parallel to the $\langle 110 \rangle$ direction of elongation. In a field of ~1 μm^2 surrounding the region exhibited here, roughly half of the ~20 platelets lying on (100) and (010) appear to outcrop on both top and bottom surfaces of the lamina containing them. This shows that their lengths parallel to the lath axis exceed 66 nm, twice the lamina thickness, as these lath axes make 60° with [110], the normal to the specimen lamina. Consequently, there is a good chance that platelets on (001) having their long axis parallel to [110] are seen with their two [110]-direction edges running straight from top to bottom of the lamina, when the direction of view of the specimen is close to [110]. The images of their edges look exactly like the images of pure edge dislocations viewed parallel to the dislocation line (as indeed they should). A platelet

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image with such properties is exhibited by one of the two (001) platelets partly covered by the overlap—the platelet in the centre of the field. It can be distinctly recognized both within the overlap region (by virtue of the clear-cut displacement of the moiré fringes) and in the single lamina region to the right of the overlap; it is best seen in Fig. 1*b*. (The lamina on the right thus corresponds to the defect-containing crystal (2), whereas that on the left is the comparison crystal (1).)

The lower platelet in the overlap region does not give a clearly defined displacement to the moiré fringes, the confusion in its fringe pattern being especially noticeable in the higher order reflection 004. We conclude that this platelet either does not outcrop at all at the interface between (1) and (2), or that the outcrop length is small relative to the platelet diameter. The perturbation to the fringe pattern arises because electrons pass through crystal that is locally tilted due to the dislocation-type strain field at the platelet periphery.

The conditions to be satisfied so that lattice displacement can be precisely derived from the moiré fringe displacement at the image of the planar defect are that both the crystal thickness and the crystal orientation are the same on either side of the plane of the defect. The former condition certainly holds in the present

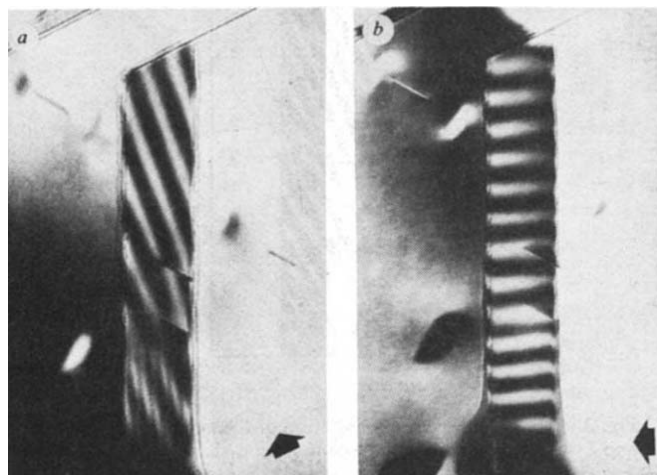


Fig. 2 Same field as in Fig. 1. Bright field images of: *a*, $\bar{1}1\bar{1}$ reflection, *b*, $\bar{1}1\bar{1}$ reflection.

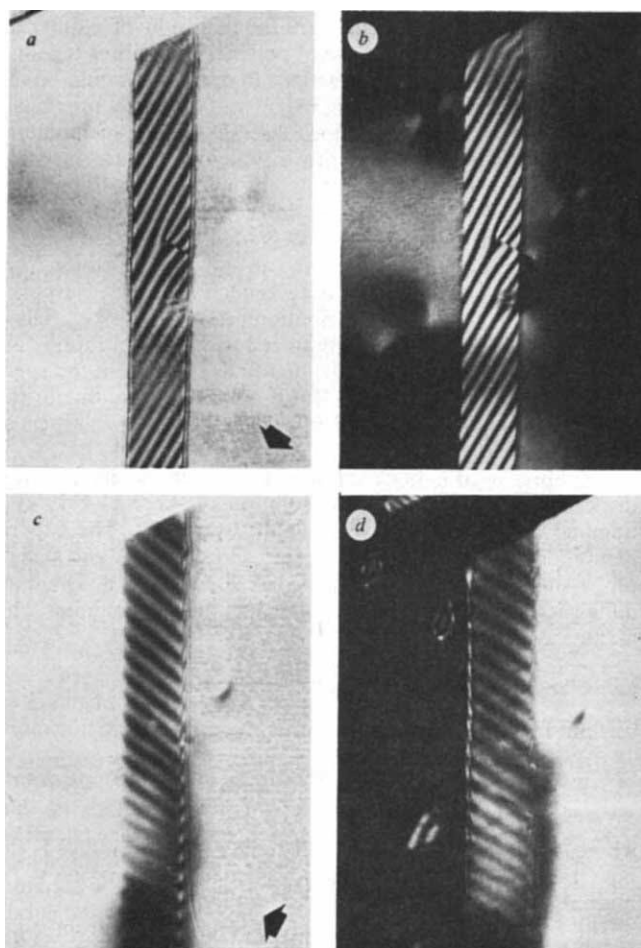


Fig. 1 Bright field and dark field electron micrographs of a platelet-containing Type Ia diamond in which moiré fringes appear where cleavage and overthrust have led to superposition of crystal laminae. The field width is $0.3\ \mu\text{m}$, the width of overlap $66\ \text{nm}$. The trace of $(\bar{1}11)$ is vertical. The micrograph print orientation reproduces a view of the image as seen on the fluorescent screen of the microscope; the specimen axis $[110]$ points towards the observer, the electron beam direction is $[\bar{1}\bar{1}0]$. Arrowheads indicate the direction of the active g vectors. *a*, 004 reflection, BF; *b*, 004 reflection, DF; *c*, $\bar{2}20$ reflection, BF; *d*, $\bar{2}20$ reflection, DF.

experiments. The latter condition applies when the platelet outcrop at the interface between crystals (1) and (2) is sufficiently extended, and is satisfied in the case of the upper platelet. In the 004 BF (Fig. 1*a*) and DF (Fig. 1*b*) images, a circuit enclosing the left-hand edge of the upper platelet shows the number of extra fringes to be slightly less than 1.5: our best estimate lies between 1.40 and 1.45. Figure 3 shows the way the fringes can be counted. For example, one may take a circuit up the right-hand margin of the overlap from fringes 3' to 1', then down along fringe 1'–1 to the left-hand margin where one proceeds to fringe 3, and then finally up along fringe 3 to the discontinuity at the platelet. The whole extra fringe is designated X; the fraction in excess of unity is assessed from the relative spacings at the platelet between fringes 2 and 3', and 3' and 3. The $\bar{2}20$ BF (Fig. 1*c*) and DF (Fig. 1*d*) fringe patterns show no detectable fringe faulting due to the platelets.

Although our moiré experiments have only included Bragg reflections from planes containing $[110]$, and are thus insensitive to translations parallel to $[110]$, it is reasonable to take Fig. 1*c* and *d* as direct evidence confirming that the matrix displacements produced by the platelet structure are perpendicular to the platelet plane. Taking $g \cdot f$ for the 004 reflection to be bracketed between the values 1.40 and 1.45, and f parallel to $[001]$, gives a bracket for the magnitude of f between $0.35\ a_0$ and $0.362\ a_0$, a_0 being the face-centred cubic lattice parameter of diamond, $0.357\ \text{nm}$.

Although Fig. 2*a* and *b* do not provide very accurate values of the displacement, their fringe shifts are quite consistent with the displacement value obtained from Fig. 1*a* and *b*. Note in Fig. 1*a* and *b* that the fringes are deviated as they approach the middle regions of the platelet. This is predictable from the strain field of the two parallel edge dislocations which are coincident with the platelet edges, and whose Burgers vector equals the platelet displacement. The calculated change in orientation of fringes 2 and 3 relative to the average fringe direction in the overlap region is $\sim 7.5^\circ$. Measurement, which can only be approximate, gives between 8° and 9° , in as good agreement as can be expected. The lower part of Fig. 2*a* shows a second fringe system. The orientation and spacing of these fringes, together with the diffraction pattern photographs, identify them as due to the $\bar{2}24$ reflection. The strong excitation of the $\bar{2}24$ reflection in this region is probably a consequence of specimen bending due to local contamination. In most of the micrographs one can see fine fringes parallel to the vertical boundaries of the overlap region. These arise partly from defocus, partly from refraction at the cleavage edge and partly from a contamination layer.

What is the nature of platelets? They are known to occur in association with nitrogen impurity in the commonest natural

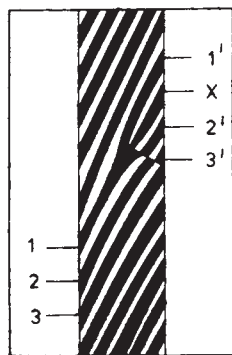


Fig. 3 Diagrammatic representation of the central part of Figs 1 and 2 showing the moiré fringe configuration as seen in Fig. 1a or b, and the numbering of fringes adopted for measuring the fringe displacement at the platelet.

Drag reduction in fibre suspension

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Spectacular reductions in turbulent friction can be obtained by adding small amounts of either long-chain polymers^{1,2}, or macroscopic fibres^{3,4} to a fluid. The polymer effect has been the more studied of the two. But one of the continuing puzzles is that measurements of the turbulent energy spectrum show no evidence of a direct eddy-polymer interaction, despite the fact that the influence of the polymers on the turbulence is so profound. Although some increase in energy may be observed at small wavenumbers (attributable to a decrease in the dissipation rate), inertial and dissipation range spectra in polymer solutions are unchanged from the newtonian result. In contrast with this situation, we have recently found very marked changes in energy spectra measured in fibre suspensions.

Although there are many similarities between the effects of the two kinds of additive, they are thought to involve different mechanisms. For example, use of polymers and fibres together can produce much larger reductions in drag than would be the case with either additive on its own⁵. We have previously reported measurements using laser-Doppler anemometry (LDA) which indicate that the turbulent structure in drag-reducing fibre suspensions is not the same as that in polymer solutions⁶ (and both, of course, differ from newtonian fluids). Furthermore, as the fibres were degraded by repeated passage through the apparatus, we observed an apparent transition from 'fibre-like' to 'polymer-like' drag reduction, as measured by mean and r.m.s. velocity distributions across the pipe⁶. These remarks apply only to typical drag-reducing concentrations. At higher polymer concentrations, one finds changes in the spectrum at large wavenumbers⁷. But these seem to be due to the onset of continuum viscoelasticity and are not *per se* an aspect of drag reduction).

The fibres used in our experiments were chrysotile asbestos, dispersed in a 0.5% aqueous solution of Aerosol OT, at a nominal concentration of 300 p.p.m. by weight. The experimental details are given elsewhere^{6,7} and the LDA and signal-processing arrangements were as described in ref. 8. The one-dimensional spectrum $E(k)$, where k is the wavenumber, and

$$\bar{u}^2 = \int_0^\infty E(k) dk$$

was put in dimensionless form using the relationship

$$\tilde{E} = \frac{E(k)}{2\bar{u}^2} R$$

where $\bar{u}^2$ is the mean-square fluctuating velocity and R is the pipe radius. The wavenumber was made dimensionless by multiplying by R .

The LDA relies on the scattering of laser light from small particles in the flow. Before discussing our results for energy spectra, we should consider the effect of light scattered from the suspended asbestos fibres (on the LDA signal). Our normal practice (for example, when working with polymer solutions) is to add scattering particles by seeding the flow with milk at a concentration of 100 w.p.p.m. The average size of the particles of fat in milk is $\sim 0.3 \mu\text{m}$ and this is small enough to follow the highest frequency turbulent fluctuations. Chrysotile asbestos occurs naturally in the form of macro fibres which are made up from basic fibrils with a mean diameter of $\sim 40 \text{ nm}$ (refs 9, 10). These macro fibres readily break down and in the specially prepared suspensions used here, the basic fibrils are preserved

diamonds (designated Type 1a). In these diamonds nitrogen is present in several non-paramagnetic aggregations, each of which has characteristic optical absorption spectra (see ref. 11 for details on optical properties and impurities in diamond). Platelets are absent in the common synthetic and the very rare natural Type 1b diamond in which nitrogen occurs in the singly substituted atomic state. Platelets are also absent in Type II diamonds, which are sufficiently pure to be free of nitrogen-dependent optical absorptions. The simplest interpretation of evidence from X-ray diffraction 'spikes' is that the lattice displacement due to platelets is close to $1/3 a_0$ normal to the platelet plane. However, considerable complications attend the performance and interpretation of the X-ray experiments¹². A platelet structure has been proposed¹³ in which pairs of nitrogen atoms replace single carbon atoms of one of the sub-lattices of the diamond structure and form a single sheet of di-nitrogens parallel to a cube plane, the N-N bonds being perpendicular to the sheet. This model displaces the diamond matrix by about $1/3 a_0$ perpendicular to the platelet plane, in accord with the X-ray evidence. Although non-nitrogen-containing platelet structures have been proposed^{14,15}, a platelet structure is now favoured in which nitrogen is at least an important constituent^{16,17}. There is scope for platelet-structure model-making, and it is important to have a strictly accurate value for the lattice displacement produced by the platelet structure. As more moiré data become available, it should be possible to narrow the uncertainty in displacement magnitude. As it stands, the present experiment provides the best and most direct measurement obtained to date.

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1. Raman, C. V. & Nilakantan, P. *Proc. Ind. Acad. Sci.* **A11**, 389-397 (1940).
2. Lonsdale, K. *Proc. R. Soc. A* **179**, 315-320 (1942).
3. Guinier, A. C. *r. Acad. Sci., Paris* **215**, 114-115 (1942).
4. Frank, F. C. *Proc. R. Soc. A* **237**, 168-174 (1956).
5. Evans, T. & Phaal, C. *Proc. R. Soc. A* **270**, 535-552 (1962).
6. Evans, T. & James, P. F. *Phil. Mag.* **11**, 113-129 (1965).
7. Mitsuishi, T., Nagasaki, H. & Uyeda, R. *Proc. Jap. Acad.* **27**, 86-87 (1951).
8. Pashley, D. W., Menter, J. W. & Bassett, G. A. *Nature* **179**, 752-755 (1957).
9. Lang, A. R. *Nature* **220**, 652-657 (1968).
10. Lang, A. R. *J. Crystal Growth* **42**, 625-631 (1977).
11. Walker, J. *Rep. Prog. Phys.* **42**, 1605-1659 (1979).
12. Lang, A. R. in *The Properties of Diamond* (ed. Field, J.) 425-469 (Academic, London, 1979).
13. Lang, A. R. *Proc. phys. Soc. Lond.* **84**, 871-876 (1964).
14. Evans, T. *Diamond Research*, 2-5 (Industrial Diamond Information Bureau, London, 1973).
15. Woods, G. S. *Phil. Mag.* **34**, 993-1012 (1976).
16. Allen, B. P. & Evans, T. *Proc. R. Soc. A* **375**, 93-104 (1981).
17. Evans, T., Qi, Z. & Maguire, J. J. *Phys. C* **14**, L379-L384 (1981).

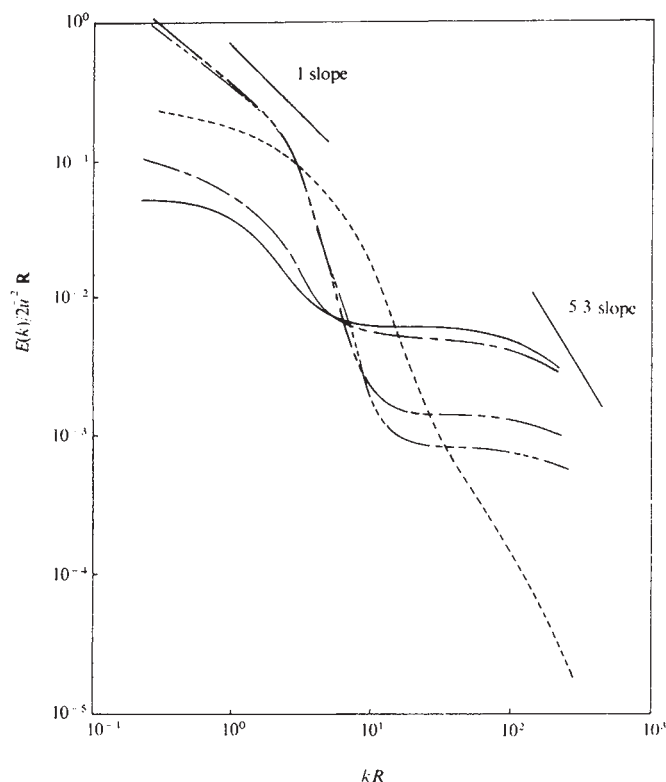


Fig. 1 One-dimensional turbulent energy spectra in a 300 p.p.m. asbestos fibre suspension at various levels of drag reduction, for $Re = 1.4 \times 10^4$; all spectra were measured at the centreline of the pipe. — Run 1, DR = 69%; --- run 2, DR = 76%; - - - - run 3, DR = 56%; - - - - - run 4, DR = 55%; results for water.

with a very high aspect ratio⁹. It is difficult to measure the length of such fibrils directly but, although there is some disagreement between statistical methods, it is clear that the mean length-to-diameter ratio of individual fibrils in an undegraded suspension is in the range 10^3 – 10^4 . Thus a contribution to the scattered light from individual fibrils might bias the LDA signal towards the lower turbulent frequencies. To assess this possibility, extensive tests were carried using various concentrations of milk, the surfactant and the asbestos fibres. In particular, we studied oscillographs of the 'burst' signals and spectra of the Doppler photocurrent. Details may be found in ref. 7 but we should mention two important conclusions here. First the Aerosol OT (presumably due to the naturally occurring micelles) was a satisfactory seeding material and milk was not needed. Second,

Table 1 Values of the eddy-fibre interaction length scale l_m as estimated from one-dimensional energy spectra

Re	DR(%)	l_m (mm)	$l_m/D^* \times 10^{-4}$
9.0×10^3	44	0.89	2.3
1.4×10^4	69	2.1	5.3
	58	1.4	3.5
	46	0.9	2.3
	38	0.84	2.1
1.4×10^4	76	2.4	6.0
	56	1.4	3.5
	55	1.2	3.0
3.2×10^4	71	1.6	4.0
	62	1.2	3.0
	58	0.95	2.4
5.3×10^4	54	1.3	3.25
	52	1.2	3.0

$D = 40$ nm.

the fibrils did not contribute to the a.c. part of the LDA signal (that is, the part that gives the fluid velocity) but only to the d.c. or pedestal level (which determines the signal-to-noise ratio). From these tests we chose a nominal fibre concentration of 300 w.p.p.m. as giving large drag reductions with satisfactory signal-to-noise ratio for the LDA.

Some results for measurements at the pipe centreline are shown in Fig. 1, for several values of drag reduction (DR) and at one Reynolds number, $Re = 1.4 \times 10^4$. In each case, an experimental 'run' consisted of one pass of the whole amount of working fluid through the apparatus at a fixed Reynolds number. Further 'runs' were further passes of the same fluid at the same Reynolds number. The Reynolds number was calculated from the pipe diameter, the bulk mean velocity and the kinematic viscosity of water.

Figure 1 shows that spectra measured in the fibre suspension have a curious 'kinked' appearance; being reduced below the newtonian spectrum level at an intermediate range of wavenumbers (roughly corresponding to the inertial range). The position and extent of the 'reduced level' region was found to depend on the amount of drag reduction. This suggested that we were observing an interaction between the turbulent eddies in a particular wavenumber (or eddy-size) range and the suspended fibrils.

To test this idea the 'reduced level' region was characterized by an interaction wavenumber k_m which was calculated by noting the two wavenumbers marking the beginning and end of this region, and taking the arithmetic mean. Hence we obtained a mean interaction length scale $l_m = k_m^{-1}$. Values of l_m are given in Table 1 for various levels of drag reduction (DR) and at four different Reynolds numbers. These values range from $l_m = 0.84$ mm (corresponding to DR = 38%) to $l_m = 2.4$ mm (corresponding to DR = 76%). Clearly we should consider what relationship (if any) exists between: (1) l_m and some length scale associated with the individual fibrils; and (2) the amount of drag reduction and l_m .

In considering the first point, note that l_m is a dynamic measurement of the length scale associated with fibrils actually interacting with the turbulent eddies. Thus there must be inponderable factors in a comparison with the length of fibrils

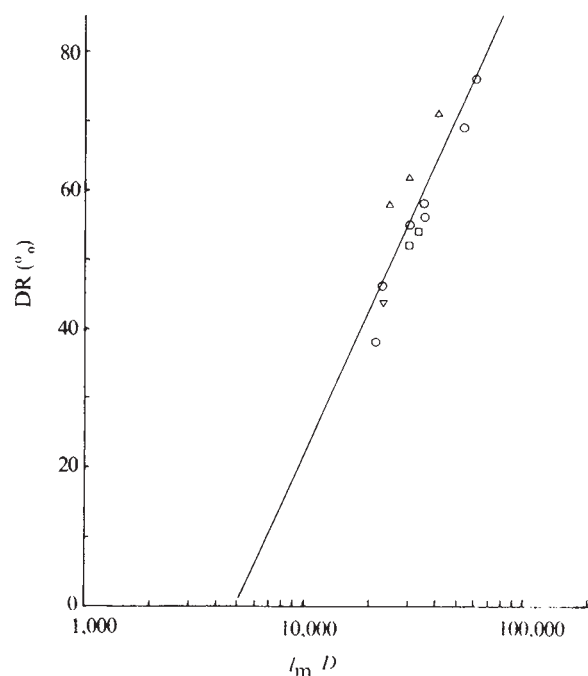


Fig. 2 Variation of percentage drag reduction (DR) with interaction length scale (l_m) in a 300 p.p.m. asbestos fibre suspension. ∇ , $Re = 0.9 \times 10^4$; $\circ$, $Re = 1.4 \times 10^4$; $\triangle$, $Re = 3.2 \times 10^4$; $\square$, $Re = 5.3 \times 10^4$.

either in a static suspension or (worse) evaporated out from such a suspension. Furthermore, there are considerable uncertainties in performing such measurements. Also the idea that the eddy interaction is with a mesh of entangled fibrils¹¹ introduces the possibility of a length scale which is larger than any individual fibril.

Nevertheless, we may expect l_m to be related to the largest fibril lengths present in any appreciable fraction and measurements of length distribution in a similar suspension suggest a mean value of 1.4 mm for the longest fibrils¹⁰. Thus we can reasonably claim that l_m is the correct order of magnitude for there to be a physical relationship to individual fibril length.

The second point was investigated by plotting values of drag reduction against the corresponding values of l_m . As can be seen from Fig. 2, the data points cluster quite well about a straight line. The variation with Reynolds number was not statistically significant and the single straight line was fitted using the least-squares method. The resulting relationship was found to be:

$$DR \times 100\% = \{0.70 \log_{10}(l_m/D) - 2.60\} \times 100\%$$

Finally, we previously remarked⁶ that the qualitative changes of turbulent structure in undegraded fibre suspensions agreed with a previous theoretical prediction¹². Note that the present results for spectra apparently support that theoretical picture. We seem to be observing a form of resonance phenomenon where the fibres are strongly excited by those turbulent eddies with spatial frequency roughly equal to the inverse of the fibril length. Thus, for once, a simple mechanistic model may well be relevant to the underlying physics of this intriguing effect.

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1. Virk, P. S. *A.I. Ch. E. J.* **21**, 625 (1975).
2. Hoyt, J. W. *Proc. 2nd Int. Conf. on Drag Reduction Paper A1*, Cambridge (BHRA, 1977).
3. Ellis, H. D. *Nature* **226**, 352 (1970).
4. Hoyt, J. W. *Naval Undersea Center Rep. TP 299*, San Diego (1972).
5. Lee, W. K., Vaseleski, R. C. & Metzner, A. B. *A.I. Ch. E. J.* **20**, 128 (1974).
6. McComb, W. D. & Chan, K. T. *J. Nature* **280**, 45 (1979).
7. Chan, K. T. J. thesis, Edinburgh Univ. (1980).
8. McComb, W. D., Allan, J. & Greated, C. A. *Phys. Fluids* **20**, 873 (1977).
9. Atkinson, A. W., Gettins, R. B. & Rickards, A. L. *Nature* **226**, 937 (1970).
10. Thew, M. T. & Anaad, J. S. *Proc. 1st Int. Conf. on Drag Reduction Paper D2*, Cambridge (BHRA, 1974).
11. Moyls, A. L. & Sabersky, R. H. *Int. J. Heat Mass Transfer* **21**, 7 (1978).
12. McComb, W. D. *Nature phys. Sci.* **241**, 117 (1973).

²¹⁰Pb in surface air at Enewetak and the Asian dust flux to the Pacific

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As part of the SEAREX programme, an air filter system and a precipitation collector were deployed at Enewetak (11°20' N, 162°20' E). A description of the air filter system and initial results on the chemical composition of the collected aerosols¹ and on the organic components² have been reported elsewhere. As part of the same programme we have measured ²¹⁰Pb (and ²¹⁰Po) in air filter and integrated monthly precipitation samples collected during 1979 to estimate the ²¹⁰Pb flux (0.15 ± 0.02 d.p.m. $\text{cm}^{-2} \text{yr}^{-1}$) and the Asian dust flux ($38 \pm 20 \mu\text{g cm}^{-2} \text{yr}^{-1}$) at this location in the Pacific.

²¹⁰Pb (22-yr half life) is a radioactive nuclide produced in the air by decay of gaseous ²²²Rn (3.8-day half life) emanating from continental soils. As ²¹⁰Pb is produced, it is scavenged by precipitation and its mean life with respect to removal from the atmosphere is ~5 days (see ref. 3 for a summary). If the primary

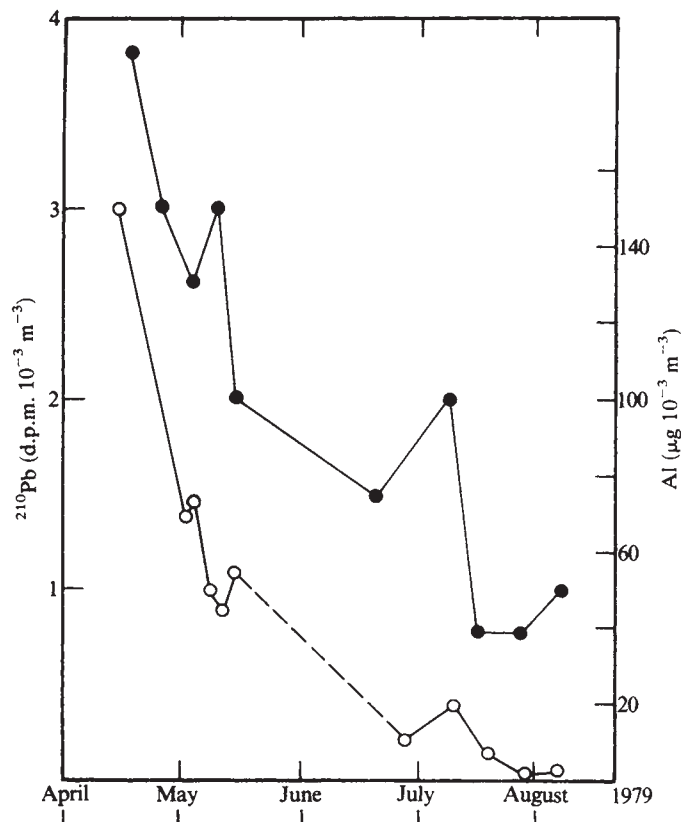


Fig. 1 ²¹⁰Pb concentrations (●) in air filter samples taken at Enewetak between April and August 1979. The Al data (○) from ref. 1 are also plotted.

source of ²¹⁰Pb in the aerosol is decay of atmospheric radon, then this short residence time precludes the presence of much ²¹⁰Po (138-day half life), the granddaughter of ²¹⁰Pb (the daughter being ²¹⁰Bi with a 5-day half life). Thus the ²¹⁰Po/²¹⁰Pb activity ratio in aerosols provides a way of determining the contribution of these nuclides from other sources with ²¹⁰Po/²¹⁰Pb activity ratios ≥ 1 , such as continental dust and ocean surface and volcanic emanations. We have used ²¹⁰Pb measurements to estimate the flux of other air-borne materials from the continents. Because its source is the Earth's surface rather than the stratosphere, as is the case for many cosmogenic and bomb-produced radionuclides, we believe that ²¹⁰Pb is a better analogue of components injected into the troposphere.

The air filter samples were taken serially; some were used for the chemical assay¹ and some for radiochemical analyses. The filters were dissolved for radiochemical analysis and ²¹⁰Po and ²¹⁰Pb determined using procedures described previously⁴. Our results are given in Table 1 and the ²¹⁰Pb data are plotted in Fig. 1. The monthly precipitation samples were collected as described by Benninger *et al.* (manuscript in preparation). Only the first seven samples of a year's collection were returned to the laboratory. The measurements (see Table 2) were made when ²¹⁰Po was virtually in equilibrium with ²¹⁰Pb. In all the air samples, the ²¹⁰Po/²¹⁰Pb activity ratios are statistically indistinguishable from 0. Thus the ²¹⁰Pb in the aerosols is derived from atmospheric ²²²Rn decay.

Although ²¹⁰Pb is derived from atmospheric radon, there is a strong correlation between the aluminium concentration (representing the soil aerosol) and the ²¹⁰Pb concentration (Fig. 1). This relationship is also observed, to some extent, for the lipid fraction of the organic component of the air², implying that the aerosol sampled is a mixture of two parts—one derived from air rich in continental mineral and plant materials and high in ²²²Rn, and the other from air containing less of these components. Duce *et al.*¹ have identified the former as dust from the Gobi Desert carried eastwards by high-level winds and

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Table 1 ^{210}Pb and ^{210}Po in air filter samples from Enewetak, April–August 1979

Date of sampling	^{210}Pb (d.p.m. 10^{-3}m^{-3})	$^{210}\text{Po}/^{210}\text{Pb}$ activity ratio
18 April	3.82 ± 0.52	—
26 April	3.03 ± 0.25	-0.02 ± 0.08
3 May	2.61 ± 0.19	-0.02 ± 0.03
10 May	3.05 ± 0.25	-0.02 ± 0.03
15 May	1.99 ± 0.18	-0.08 ± 0.03
20 June	1.49 ± 0.05	0.14 ± 0.11
8 July	2.00 ± 0.08	0.02 ± 0.28
16 July	0.786 ± 0.035	-0.26 ± 0.04
26 July	0.778 ± 0.019	0.17 ± 0.07
4 August	1.01 ± 0.04	0.05 ± 0.07
Tower blanks (filter carried up the sampling tower in exposed conditions)		
10 May	0.106 ± 0.027	-0.09 ± 0.10
26 July	0.045 ± 0.008	-0.05 ± 0.18

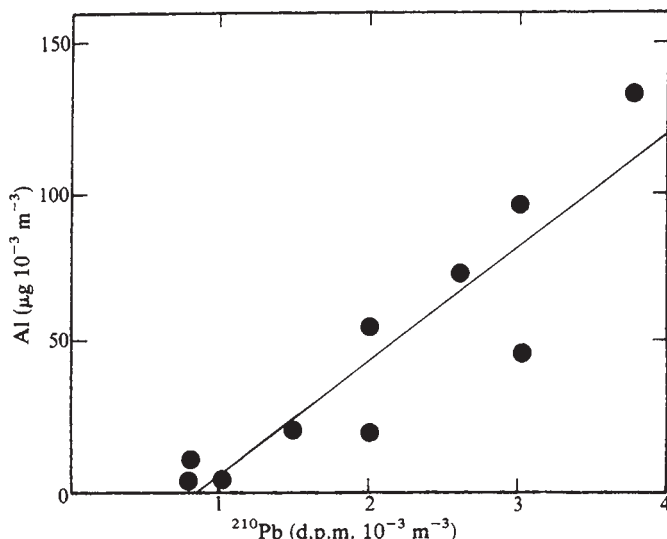
Sampling date represents the date corresponding to the midpoint of the collection period. Errors are 1σ counting errors for all values given.

injected below the trade wind inversion east of Enewetak. The source of the air with low concentrations of all components must be the east, because of the predominantly easterly circulation.

Comparison of our Enewetak air filter results with observations made at Hawaii in 1971⁵ shows considerably higher values of ^{210}Pb at Hawaii. Between 15 May and 20 June 1979 at Enewetak, the concentration of ^{210}Pb was 1.5 – 2 d.p.m. 10^{-3}m^{-3} . Between 30 May and 22 June 1971 at Hawaii, the values below the trade wind inversion were 3.4 – 19.4 d.p.m. 10^{-3}m^{-3} with a mean of 9.3 , and above were 25.6 – 93.4 d.p.m. 10^{-3}m^{-3} with a mean of 46 . Therefore the mean ^{210}Pb concentration in surface air at Hawaii is about five times greater than at Enewetak.

The use of ^{210}Pb to determine fluxes of continentally derived material requires an independent measure of the ^{210}Pb flux at the site. Direct measurements are not yet available for Hawaii but we have made a global model for the ^{210}Pb flux which can be used to estimate the flux around Hawaii³. This value is ~ 1 d.p.m. ^{210}Pb $\text{cm}^{-2}\text{yr}^{-1}$. The ^{210}Pb flux at Enewetak has been measured using the monthly precipitation collections made during the year when the aerosol samples were taken. Although several precipitation samples were lost in transit we believe we can still reasonably estimate the flux from the recovered samples. Table 2a shows that although the monthly fluxes show variations, these are small and independent of season. The average of all the monthly fluxes yields an estimate of the annual ^{210}Pb flux of 0.15 (± 0.02) d.p.m. ^{210}Pb $\text{cm}^{-2}\text{yr}^{-1}$ —a factor of seven lower than the Hawaii model flux estimate.

The apparent relationship between the mean concentration of ^{210}Pb in the air over a month's sampling and the monthly

**Fig. 2** Al versus ^{210}Pb in air filter samples from Enewetak 1979.

precipitation flux of ^{210}Pb is shown in Table 2b. The relationship can be used to estimate the mean annual concentration of ^{210}Pb (C) in the air based on the average ^{210}Pb flux (F). (The regression equation is $C[\text{d.p.m. } ^{210}\text{Pb } 10^{-3}\text{m}^{-3}] = 4.7 - 21.4 \times F[\text{d.p.m. } ^{210}\text{Pb } \text{cm}^{-2}\text{yr}^{-1}]$.) A mean ^{210}Pb flux of 0.15 d.p.m. $\text{cm}^{-2}\text{yr}^{-1}$ corresponds to a mean air concentration of 1.5 d.p.m. 10^{-3}m^{-3} .

It is evident from Table 2b that the ratio of ^{210}Pb flux to air concentration, which has the dimensions of a velocity (= 'total deposition velocity'), varies as a function of season. The dry season is characterized by a velocity of 1 cm s^{-1} and the wet season by 4 cm s^{-1} . The total deposition velocity at Hawaii between 30 May and 22 June 1971 is $\sim 3\text{ cm s}^{-1}$. These values are comparable with that of $\sim 4\text{ cm s}^{-1}$ for the region around Bermuda (L. K. Benninger *et al.*, in preparation) determined for the same time of year. All these oceanic sites have higher values than continental sites (ref. 3 and L. K. Benninger *et al.*, in preparation). The air above the trade wind inversion is the source of most of the ^{210}Pb flux at subtropical oceanic sites because this air has trajected far over continents. When the aerosols above the trade wind inversion are supplied to the inversion, they are efficiently scavenged by precipitation resulting in lower ^{210}Pb concentrations below the trade wind inversion than above (see ref. 5). This causes the total deposition velocity, measured at sea level, to be higher than that measured above the trade wind inversion. Strong mixing over continents tends to homogenize the lower air ^{210}Pb concentration, thus yielding a lower total deposition velocity. We believe that the high deposition velocity given by the ^{210}Pb data at the ocean surface should be used to obtain fluxes of material derived from

Table 2 a, ^{210}Pb in rain at Enewetak (April–November 1979) and b, relationship between ^{210}Pb in air and ^{210}Pb precipitation flux

<i>a</i> Collection period (1979)		Amount of rain (kg)	^{210}Pb (d.p.m. kg^{-1})	^{210}Pb flux (d.p.m. $\text{cm}^{-2} \text{yr}^{-1}$)	
23 April–20 May		0.138	8.72 ± 0.41	0.089	
20 May–17 June		1.045	2.11 ± 0.08	0.157	
18 June–18 July		0.762	3.43 ± 0.11	0.173	
19 July–20 August		3.795	0.68 ± 0.03	0.160	
21 August–26 September		1.301	1.20 ± 0.06	0.086	
26 September–19 October		2.491	1.21 ± 0.04	0.261	
19 October–28 November		2.378	1.05 ± 0.05	0.124	
<i>b</i>					
	Season	No. of air measurements	Mean [^{210}Pb] in air (d.p.m. per 10^3 m^3)	^{210}Pb precipitation flux (d.p.m. $\text{cm}^{-2} \text{yr}^{-1}$)	Total deposition velocity (cm s^{-1})
April 18–May 15		5	2.9	0.089	1.0
June 20–July 16		3	1.4	0.173	4.1
July 26–August 4		2	0.89	0.160	4.1

For the rain samples ^{210}Pb was calculated from ^{210}Po measured 13–22 months after collection, assuming no initial ^{210}Po and ^{210}Bi activity = ^{210}Pb activity. Area of collector for ^{210}Pb flux = 183.4 cm^2 .

continents on the premise that the same processes apply to all components derived from the continental surface.

By combining the measured ^{210}Pb flux and the aerosol ^{210}Pb and Al concentrations, we can directly estimate the flux of air-borne dust at Enewetak. Comparing the Al and ^{210}Pb curves of Fig. 1 shows that the trends are coherent and that a correlation between the two variables exists. As the filter samples analysed for ^{210}Pb and Al were taken serially, it is possible, by interpolation, to estimate the Al concentration corresponding to each of our ^{210}Pb measurements using Fig. 1. The resultant plot is shown in Fig. 2. A linear regression of Al against ^{210}Pb yields a slope of $38 \mu\text{g Al per d.p.m. } ^{210}\text{Pb}$ with a y intercept of $-32 \mu\text{g Al}$ and a correlation coefficient of 0.91. We believe this represents a mixing curve between high ^{222}Rn (and therefore ^{210}Pb), high Al air and low ^{222}Rn , low Al air. The $^{222}\text{Rn}/\text{Al}$ ratio of each of the end members is obviously different as the regression line of Fig. 2 does not go through the origin.

Using the relationship shown in Fig. 2, we can determine the atmospheric flux of aluminium if we know the flux of ^{210}Pb , $F_{210\text{Pb}}$, and the mean ^{210}Pb concentration in the air, $[^{210}\text{Pb}]$, as follows:

$$F_{\text{Al}} = \left(S + \frac{y}{[^{210}\text{Pb}]} \right) F_{210\text{Pb}}$$

where S is the slope ($38 \mu\text{g Al per d.p.m. } ^{210}\text{Pb}$) and y the y intercept in units of $\mu\text{g Al } 10^{-3} \text{ m}^{-3}$ ($= -32$).

Assuming that the mean concentration of ^{210}Pb in Enewetak air is $\sim 1.5 \text{ d.p.m. } 10^{-3} \text{ m}^{-3}$ during wet and dry seasons as discussed above, the Al flux at Enewetak is $2.5 \mu\text{g Al cm}^{-2} \text{ yr}^{-1}$. An aluminium concentration of the dust component of 6.5% as used by Duce *et al.*¹ corresponds to $38 \mu\text{g dust cm}^{-2} \text{ yr}^{-1}$ for Enewetak. The uncertainties in all the components of the calculation are significant. We estimate therefore that the (1σ) uncertainty could be as much as $\pm 20 \mu\text{g dust cm}^{-2} \text{ yr}^{-1}$.

Duce *et al.*¹ estimate the annual flux of dust to the region around Enewetak by multiplying the measured monthly flux for late April and May ($4 \mu\text{g cm}^{-2}$ per month) by 3–4 on the basis of dust frequency information. This yields a flux of ~ 12 – $16 \mu\text{g cm}^{-2} \text{ yr}^{-1}$, a factor of 2–3 lower than our estimate.

A calculation of wind-blown detrital flux for the latitude of Hawaii can be made in a similar fashion to that for Enewetak. If we use the model ^{210}Pb flux for Hawaii, and assume that the same air mass end members as at Enewetak are involved, a mean ^{210}Pb atmospheric concentration of $9.3 \text{ d.p.m. } 10^{-3} \text{ m}^{-3}$ yields a flux of $35 \mu\text{g Al cm}^{-2} \text{ yr}^{-1}$, equivalent to a dust flux of $530 \mu\text{g cm}^{-2} \text{ yr}^{-1}$ (assuming 6.5% Al). This is about 14 times higher than that predicted for the latitude of Enewetak and shows the dominant influence, at the latitude of Hawaii, of the westerlies carrying radon and dust from Asia. Both Enewetak and Hawaii are far enough from the land sources that the dust will be dominated by the small sizes and therefore subject to removal by the same processes as those for indigenously produced ^{210}Pb . Based on our calculated dust fluxes, the sediment accumulation rates should be higher at the latitude of Hawaii and northwards than at the latitude of Enewetak. This does not seem to be the general case although there are few data for this region^{6,7}, implying that either our assumptions about the broad applicability of the relationship of Fig. 2 are invalid or there has been redistribution of sediments after deposition from the atmosphere but before final accumulation. The evidence for a strong atmospheric flux from the Gobi Desert supports the latter alternative. A more detailed analysis of sediment accumulation rates in the North Pacific is needed.

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1. Turekian, K. K., Kharkar, D. P. & Thomson, J. *Final Rep. ARPA Order no. 1793 contract N0014-67-A-0097-0022*.
2. Moore, H. E., Poet, S. E. & Martell, E. A. *J. geophys. Res.* **79**, 5019–5024 (1974).
3. Goldberg, E. D. & Koide, M. *Geochim. cosmochim. Acta* **26**, 417–450 (1962).
4. Cochran, J. K. thesis, Yale Univ. (1979).

Identification of polychlorinated dibenzofurans in environmental samples

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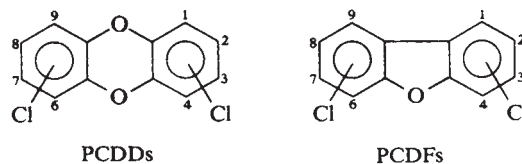
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The polychlorinated dibenzofurans (PCDFs) are tricyclic aromatic compounds, which in chemical and toxicological respects are very similar to polychlorinated dioxins (PCDDs). The 2,3,7,8-tetra-, 1,2,3,7,8- and 2,3,4,7,8-penta-CDFs are extremely hazardous



compounds, they can be compared with the extremely toxic 2,3,7,8-tetra-CDD (ref. 1). PCDFs have been identified in PCBs at levels of 1–10 p.p.m., the major peaks being 2,3,7,8-tetra- and 2,3,4,7,8-penta-CDF (refs 2–4). A large number of PCDFs and PCDDs occur as contaminants in chlorophenols^{5–7}, and they have also been recognized and identified in fly ash and other incineration products at the p.p.b.–p.p.m. level^{8–11}. In 1968, 1,200 people in Japan were poisoned (Yusho disease) by a cooking oil contaminated by PCB¹² and more than 50 PCDF isomers¹³. Liver samples from exposed patients were found to contain a highly reduced number of PCDFs, apparently many isomers were excreted or metabolized¹⁴. We report here the identification of a series of PCDF isomers in environmental samples.

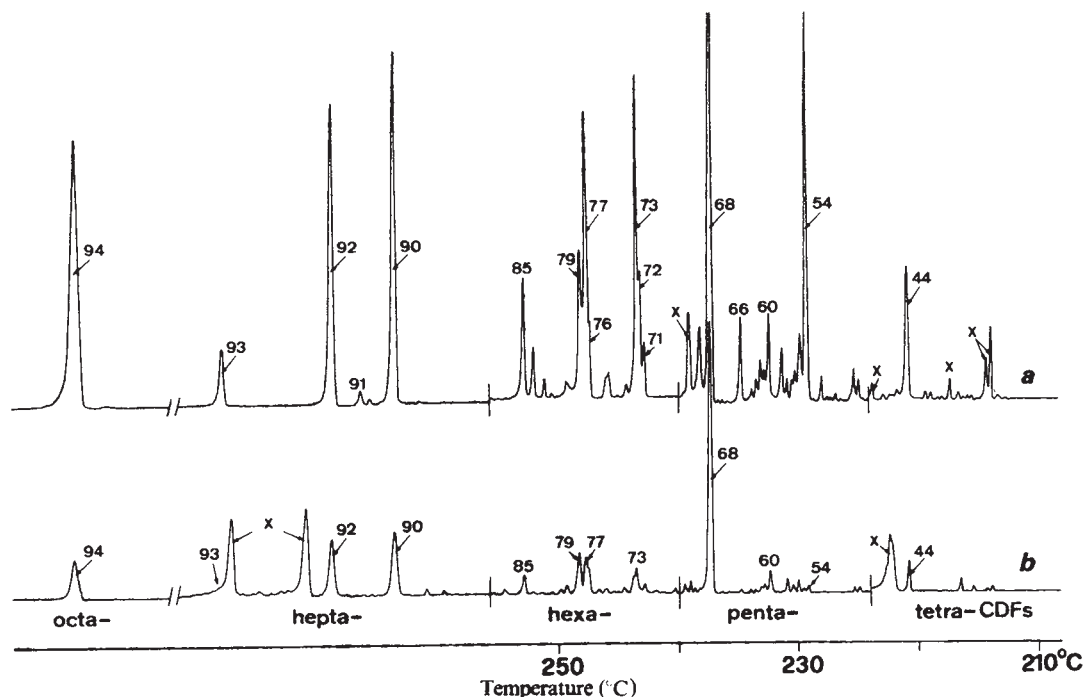
Fat from the snapping turtle (*Chelyda serpentina*) obtained from the Hudson River (USA) and grey seal (*Halichoerus grampus*) from the Gulf of Bothnia (Haparanda, Sweden) were processed for PCDF analyses in two series of sequential chromatographic processes¹⁵. The turtle fat contained 750 p.p.m. of PCBs and the seal fat PCB concentration was ~ 100 p.p.m.

Fat tissue (50–100 g) ground with sodium phosphate was applied to the first chromatographic system which contains a segment of potassium silicate and a segment of silica gel directly followed by a column containing carbon dispersed on glass fibres. The carbon adsorbs most multi-ring aromatic compounds and the major portion of biological co-extractives are not retained. The PCDFs, PCDDs and similar chemicals are then removed from the carbon by reverse elution with toluene. The toluene is removed and the sample is redissolved in hexane and applied to two columns in tandem. The first column contains both sulphuric acid dispersed on silica gel and caesium silicate. The eluate from this column passes directly into the second column that contains alumina.

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1. Duce, R. A. *et al. Science* **209**, 1522–1524 (1980).
2. Gagosian, R. B., Peltzer, E. T. & Zafiriou, O. C. *Nature* **291**, 312–314 (1981).
3. Turekian, K. K., Nozaki, Y. & Benninger, L. K. *A. Rev. Earth planet. Sci.* **5**, 227–255 (1977).

Fig. 1 NCI Mass fragmentograms (m/e 306, 340, 374, 408 and 442) of *a*, Hudson turtle and *b*, Baltic seal sample showing elution of tetra-, penta-, hexa-, hepta- and octa-CDFs; 50 m OV-17 glass capillary column. For peak identifications see Table 1; X denotes components other than PCDFs.



The purified extracts were directly used for GC-MS analysis. Aliquots (2 μ l) corresponding to 0.1 and 0.8 g of fat of the snapping turtle and grey seal, respectively, were injected into 50-m glass capillary columns (OV-17 and Silar 10 c) leading directly into the ion source of a mass spectrometer operating in the negative chemical ionization (NCI) mode using methane as reactant gas. PCDFs exhibit intense negative molecular ions (M^-). Some fragmentation occurs through addition of H and loss of Cl with formation of ($M-34$) $^-$ ions.

Mass specific detection (mass fragmentography) was used to detect PCDFs in these samples by selective monitoring of ($M+2$) $^-$ ions at m/e 306 (tetra-), 340 (penta-) and so on. More highly chlorinated PCDFs give response at the m/e values for the less highly chlorinated species due to the ($M-34$) $^-$ fragmentation. Quantification was based on peak height measurements and comparison with known quantities of authentic standards (2,3,7,8-tetra- and octa-CDF). Minimum detectability was 0.2 pg and 0.5 pg for 2,3,7,8-tetra- and octa-CDF, respectively. The same response for all isomers of a particular PCDF was assumed, because quantitative standards are not available. Responses for penta-, hexa- and hepta-CDFs were interpolated from the responses of tetra- and octa-CDF.

Complete NCI mass spectra (m/e 80–500, 1.4 s per scan) were recorded for the major peaks. Identification of a PCDF was based on the proper M^- ions and intensities of the ion clusters due to the Cl isotopes. Isomer identifications were based on the retention times on both OV-17 and Silar 10 c capillary columns and comparison with retention times of authentic standards.

In spite of differences in the geographical and biological origin, the samples show good correspondence. Figure 1a shows the mass fragmentograms of the turtle fat. Using the requirements discussed above, 18 different PCDF isomers could be identified (see Table 1). The total amount of PCDFs was estimated as 3 p.p.b. (Table 2). Of the individual PCDF isomers, the amount of 2,3,7,8-tetra-CDF was 45 p.p.t. and that of 2,3,4,7,8-penta-CDF was 620 p.p.t.

Figure 1b shows the mass fragmentogram of the seal fat, and the isomers identified are given in Table 1. These levels were much lower than in the turtle fat, the total amount being 40 p.p.t. The amount of 2,3,7,8-tetra-CDF was 1 p.p.t. and that of 2,3,4,7,8-penta-CDF was 15 p.p.t.

In the two samples the major PCDFs were 2,3,7,8-tetra-, 1,2,4,7,8- and 2,3,4,7,8-penta-, 1,2,4,6,7,8-, 1,2,3,4,7,8-, 1,2,3,6,7,8- and 2,3,4,6,7,8-hexa-, 1,2,3,4,6,7,8- and

1,2,3,4,6,8,9-hepta- and octa-CDF (see Fig. 1, Table 2). These isomers were also found to be the major PCDFs in commercial PCBs such as Aroclor 1254 and 1260 (ref. 4). However, in these commercial products some additional PCDFs were present; in the environmental samples these isomers (1,2,3,4,8- and 2,3,4,6,7-penta- and 1,2,3,4,6,7-hexa-CDF) were absent or present at much reduced levels. No such isomers were found in the study of the post-exposure tissue samples of Yusho patients, excretion or metabolism of these isomers was suggested¹⁴.

The major PCDFs in commercial pentachlorophenols are 1,2,4,6,8-penta- and 1,2,4,6,8,9-hexa-CDF (refs 5–7). The former isomer was not found at all, and the latter was found at a very low level in these environmental samples. The isomer distribution of the PCDFs was also different from that reported in fly ash or PCB pyrolysates—notably differences in the

Table 1 PCDF isomers found in fat samples of the snapping turtle (Hudson River) and grey seal (Gulf of Bothnia)

PCDF-isomer	Peak no.*	Turtle	Seal
2,3,7,8-tetra-	44	++§	+§
1,2,4,7,8-penta	54	++§	(+)
1,2,3,7,8-penta-	60	(+)	(+)
1,2,6,7,8-penta-	66	(+)	
2,3,4,7,8-penta-	68	++++§	+++§
1,2,3,4,6,8-hexa	71	+	
1,3,4,6,7,8-hexa-	72	+	
1,2,4,6,7,8-hexa-	73	++§	+
1,2,4,6,8,9-hexa-	76	(+)	(+)
1,2,3,4,7,8-hexa-	77	+++§	+
1,2,3,4,6,7-hexa-	78	(+)	
1,2,3,6,7,8-hexa-	79	++§	+
2,3,4,6,7,8-hexa-	85	+	(+)
1,2,3,4,6,7,8-hepta-	90	+++§	++§
1,2,3,4,6,7,9-hepta-	91	(+)	
1,2,3,4,6,8,9-hepta-	92	+++§	++§
1,2,3,4,7,8,9-hepta-	93	+	
octa	94	++++§	++§

* Peak no. refers to Fig. 1a, b.

(+) Trace amounts possibly present. + Minor peak. ++ Middle peak. +++ Major peak. ++++ Dominating peak.

§ Identification by MS and retention times on two columns.

|| Identification by retention times on two columns only.

Table 2 Amounts of PCDFs found in fat samples of a snapping turtle (Hudson River) and a grey seal (Baltic)

	Turtle (pg per g)	Seal (pg per g)
Tetra-CDFs	45	1
Penta-CDFs	820	15
Hexa-CDFs	700	8
Hepta-CDFs	1,000	10
Octa-CDF	350	3
Total PCDFs	3,000	40

hexa- and hepta-CDF range¹¹. The 1,2,3,4,6,8-hexa- and 1,2,3,4,6,7,9-hepta-CDFs are both dominant in the fly ash samples and PCB pyrolysates while they are absent or nearly absent in these fat samples. In contrast 1,2,3,4,6,8,9-hepta-CDF, a major isomer in the fat samples, is only a minor isomer in fly ash and PCB pyrolysate. Consequently the major source of the PCDFs in the fat samples of the snapping turtle and grey seal is probably a direct contamination by PCBs.

Earlier attempts to identify PCDFs in the environment were unsuccessful due to the lack of sensitivity of the analytical techniques¹⁶ and the lack of selective contaminant enrichment procedures¹⁵. To our knowledge this is the first identification of the highly toxic 2,3,7,8-tetra- and 2,3,4,7,8-penta-CDF in environmental samples.

Received 12 March; accepted 10 June 1981.

- Moore, J. A., McConnell, E. E., Dalgard, D. W. & Harris, M. W. *Ann. N.Y. Acad. Sci.* **320**, 151–163 (1979).
- Bowes, G. W., Mulvihill, M. J., Simoneit, B. R. T., Burlingame, A. L. & Risebrough, R. W. *Nature* **256**, 305–307 (1975).
- Albro, P. W. & Parker, C. E. *J. Chromatogr.* **169**, 161–166 (1979).
- Rappe, C. & Buser, H. R. in *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (ed. Kimbrough, R. D.) 41–76 (Elsevier, Amsterdam, 1980).
- Buser, H. R. thesis, Univ. Umeå (1978).
- Buser, H. R. & Bosshardt, H.-P. *J. Ass. Off. analyt. Chem.* **59**, 562–569 (1976).
- Rappe, C., Garå, A. & Buser, H. R. *Chemosphere* **7**, 981–991 (1978).
- Olie, K., Vermeulen, P. L. & Hutzinger, O. *Chemosphere* **6**, 455–459 (1977).
- Bumb, R. R. *et al. Science* **210**, 385–390 (1980).
- Buser, H. R., Bosshardt, H.-P. & Rappe, C. *Chemosphere* **7**, 165–172 (1978).
- Buser, H. R., Bosshardt, H.-P., Rappe, C. & Lindahl, R. *Chemosphere* **7**, 419–429 (1978).
- Higuchi, K. (ed.) *PCB Poisoning and Pollution*, 1–179 (Academic, Tokyo, 1976).
- Buser, H. R., Rappe, C. & Garå, A. *Chemosphere* **7**, 439–449 (1978).
- Rappe, C., Buser, H. R., Kuroki, H. & Masuda, Y. *Chemosphere* **8**, 259–266 (1979).
- Stalling, D. L., Petty, J. D., Smith, L. M., Rappe, C. & Buser, H.-R. *Proc. Workshop on Impact of Chlorinated Dioxins and Related Compounds on the Environment*, Rome, 22–24 October 1980 (Pergamon, Oxford, in the press).
- Zitko, V., Hutzinger, O. & Choi, P. M. K. *Envir. Hlth Perspect.* **5**, 47–50 (1973).

Indigenous ¹³C-NMR structural features of soil humic substances

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¹³C-NMR has recently been used to characterize the structure of solid humic and fulvic acids^{1–7}. However, these studies all used the classical NaOH extraction followed by acid precipitation (humic acids precipitate, fulvic acids are acid and base soluble) to isolate humic substances. There has been concern^{8–10} over the chemical alteration of such isolated acids. The studies reported here describe the effects of mild extraction techniques compared with the classical isolation procedure. Fractions were characterized by IR spectroscopy and ¹³C-NMR. In addition, IR and ¹³C-NMR spectra of hemicellulosic components, extracted by the classical procedure from cellulose, are compared with spectra of humic and fulvic acids isolated by the same procedure. These spectra elucidate the effect of extractants on aromaticity, paramagnetic coextractives effects on spectra quality, and spectral evidence for a cellulose component as an integral part of humic substances.

Numerous attempts have been made to devise a structural formula representative of humic and fulvic acids¹¹. Much of the difficulty may lie in the techniques used to isolate humic substances; these are often so severe (0.5 M NaOH extraction, concentrated HCl precipitation, HCl/HF purification) as to preclude the isolation of indigenous compounds.

For complex compounds, such as humic substances, the molecules may consist of rapid and mobile regions in which only long transversal relaxation times, T_2 , predominate to be observable as broad linewidths in a high resolution spectrum. Longitudinal relaxation times, T_1 , will be even longer as T_1 is always greater than T_2 . Consequently, caution is required when deriving concentrations of carbon classes from intensities of the signals or in deducing from the absence of a signal the essence of a specific structure. Low signal-to-noise ratios are common in humic spectra due to multiple causes of line broadening.

Humic acids extracted at different pH values exhibited an increase in aromatic protons (¹H-Fourier-transformed (FT)-NMR) as pH decreased from 14.0 to 4.5; however, aliphatic protons were always most abundant at any pH (65–81%)¹². Additional studies have emphasized differences in ¹³C-NMR spectra based on nature of the extractant used to isolate humic and fulvic acids^{4,6,13}, and interpretation of chemical shifts especially in the 110–160 p.p.m. range. This range is classically assigned to aromatic carbon absorptions but there is evidence^{14–16} that alkenic carbon resonances may be present.

The isolation^{17–20} of humic-like substances from plants has previously been reported^{18–21}. Humic acids were extracted¹⁰ from humifying leaves and clover roots; IR spectra were almost identical to fulvic and humic acids isolated from soil.

Hemicelluloses are an important constituent of raw organic matter, especially in mineral soils (35% in wheat straw²²). They are typically extracted from wood with 40% NaOH followed by addition of acetic acid to the filtrate to yield the precipitate hemicellulose²³. Hemicellulose was first used to describe a product obtained from cereals with dilute alkali treatment and precipitation of the extract with acid²⁴. The product was easily hydrolysed to give a mixture of sugars.

Cellulose contains hemicelluloses which are alkali soluble and precipitable with acid. Acid treatment can degrade the polysaccharides in hemicelluloses to furfurals. Glucuronic and galacturonic acids (associated with hemicelluloses) are degraded by 12% HCl to xylose and arabinose respectively; both of these subsequently degrade to furfural in the presence of acid²⁵.

We consider the non-destructive isolation, purification and analysis of humic acids from an agricultural soil. A precursor of humus substances was also analysed using the classical isolation procedure.

Soil (orthic black chernozem, 38% sand, 30% silt, 32% clay, 5.4% organic carbon) was extracted with 0.5M NaOH (aq) solution purged with nitrogen in a brown glass bottle and shaken for 6 h. Samples were centrifuged for 30 min at 13,000 r.p.m. The supernatant was filtered and the filtrate slowly acidified to pH 1.5 with concentrated HCl. The humic acid precipitate (I) was centrifuged; the pellet was dissolved in weak alkali and ultrafiltered on a Diaflo UM2 membrane (<1,000 molecular weight excluded) in a stirred cell to desalt and concentrate the humic solution. When the filtrate reached a pH of 6.5 and the volume was reduced to 500 ml, the retentate was shell frozen and lyophilysed. Humic acids were stored in a desiccator under vacuum. Fulvic acid was prepared from a separate soil sample using 0.5 M NaOH as described above, except that the alkali-acid soluble supernatant obtained after the humic acid centrifugation was ultrafiltered (UM2-Diaflo) and lyophilysed.

Aqueous 0.02 M Na₄P₂O₇ was neutralized to pH 7 and used to extract the same soil to yield humic acid II. The second Na₄P₂O₇ extract was treated using acetone (1:1) to salt-out the humic acids (III) instead of acid precipitation. This precipitate (III) did not have to be dispersed with base for ultrafiltration as it was the sodium salt. Yields of humic acids were, 0.93 g (I), 0.57 g (II), and 1.47 g (III) per 500 g of soil respectively.

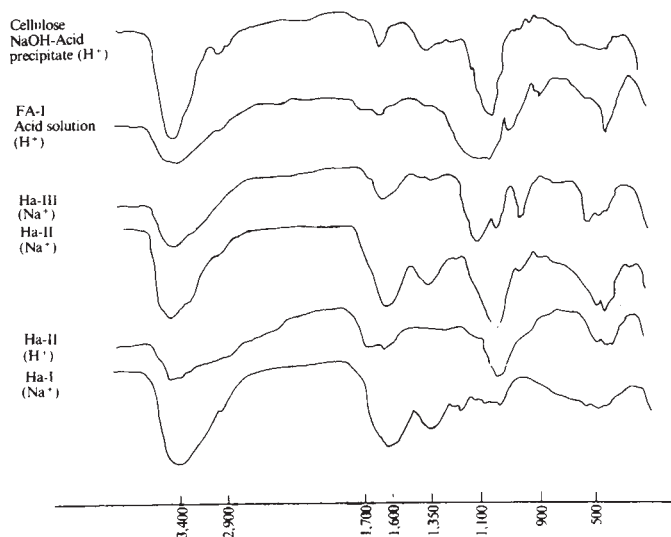


Fig. 1 Composite IR spectra of humic and fulvic acids and a cellulose component isolated in these studies (CM^{-1}).

The ratio of 1:1 acetone/water extract used for preparation of humic acid III was the minimum amount of acetone necessary for precipitation to occur. The supernatant from humic acid II did not yield any visible precipitate on addition of acetone (1:1) and humic acid III supernatant did not yield a precipitate on acidification to pH 1–2; therefore, the precipitates may be taken in each case to represent humic acids and not just a fraction of these.

IR spectra (Fig. 1) were generally diffuse and lacked coherent, well-defined resolution. However, at equal concentrations of humic acid analysed, absorption in the region of aromatic carbon ($1,620\text{ cm}^{-1}$ (ref. 26), and $1,690\text{ cm}^{-1}$ (ref. 27)) decreased in intensity as: $\text{I} > \text{II} > \text{III}$. The fulvic acid IR spectra was most similar to humic acid III. This indicates the possible deformation of indigenous structure due to the acidification step.

^{13}C -FT-NMR spectra for humic acids, I, II, III, and a NaOH-extracted humic acid, precipitated, and exhaustively de-ashed humic acids (I–IV) are shown in Fig. 1. Aromatic/alkenic

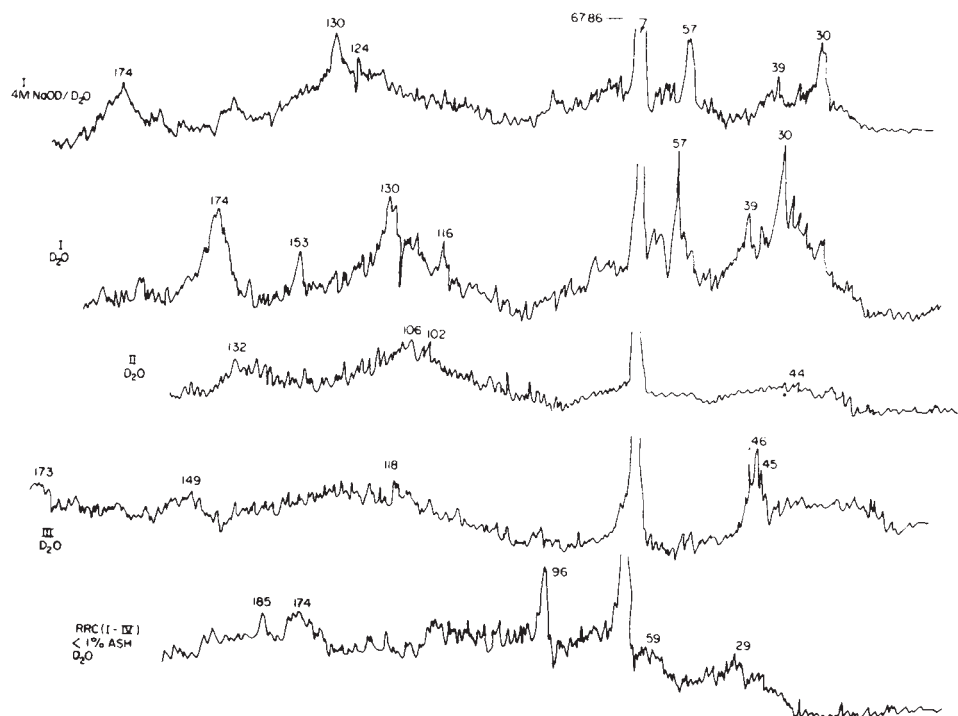
absorption in the 100–160 p.p.m. region increases ($\text{I} > \text{II} > \text{III}$) as the harshness of the extraction procedure increases, that is, when NaOH and HCl are used. This corroborates the IR evidence, which illustrated the same effect.

A humic acid, prepared from a Red River Clay soil (RRC I–IV, 40% sand, 16% silt, 44% clay, 4.4% organic carbon), with a very low ash content (0.1% ash) yielded a spectrum (Fig. 2) not much different in terms of broad and low intensity absorption when compared with higher ash content humic acids. The spectrum was most similar to that for fulvic acid (Fig. 3) and cation analysis showed these two humic substances to have the lowest concentration of Cu^{2+} and Fe^{3+} (Table 1). Although the concentrations were much lower than the other humic acids (I–III) they were still high enough to cause profound peak broadening (due to paramagnetic ion-induced relaxation). Concentrations as low as 6 p.p.m. of Cu^{2+} in a glycylglycine sample causes absorptions to almost disappear²⁸. Cu^{2+} concentrations were 52 and 114 p.p.m. for the fulvic acid and de-ashed humic acid respectively.

There were only slight differences between the spectra of humic acid I in D_2O solution compared with that in a 4 M NaOD solution (Fig. 2), indicating the lack of observable (^{13}C -NMR) alteration in the humic acid during radiation ($\approx 48\text{ h}$). Any alkaline degradation would have already occurred during the preparation of I.

For the humate extracted by 0.5 M NaOH, the peaks at 30.05, 38.96 and 57.52 p.p.m. fall in the range of aliphatic carbon absorption. According to Abraham and Loftus²⁹, possible configurations for 30.05 p.p.m. would be $\text{R}-\text{CH}_2-\text{R}$ or $\text{R}_3\text{C}-\text{R}$, while the bands at 38.96 and 57.52 p.p.m. could reflect $\text{R}_2\text{CH}-\text{R}$. The major peaks at 116.26 and 130.46 p.p.m. could be due either to alkenic or aromatic carbons. Early workers in ^{13}C -NMR assigned the alkenic carbons to an absorption between 120 and 155 p.p.m., as opposed to a much broader aromatic region³⁰. ^{13}C -NMR of humic substances had generally assigned the area 110–160 p.p.m. to aromatic carbon^{4,31}; however, more recently the area of absorption of alkenic carbons has widened such that they cover a wider band than aromatic systems²⁹. Thus absorption in the 110–160 p.p.m. range could belong to either aromatic or alkenic carbons^{32,33}. Positive identification of two C–H aromatic carbons at 130 and 128 p.p.m. in a humic acid have been reported³⁴ and supported by the observation that on loss of proton decoupling the peaks split into doublets. Thus, in soil humic matter, there are some

Fig. 2 Composite of humic acid ^{13}C -NMR spectra isolated by various extraction methods. ^{13}C -NMR spectra were obtained on a Bruker WS 900S Multinuclear NMR spectrometer operating at 22.63 MHz (35 °C). Free induction decays were digitized, accumulated and a Fourier transformation performed by computer. D_2O lock and full proton decoupling were used during an accumulation of 80,000–100,000 scans using 90° pulse and total pulse delay and acquisition time of 1.589 s, pulse width = 14 μs , internal standard deuterio-dioxane (67.86 p.p.m.), chemical shifts reported in p.p.m. resolution = $\pm 10\text{ Hz}$ ($\sim 5\text{ p.p.m.}$).



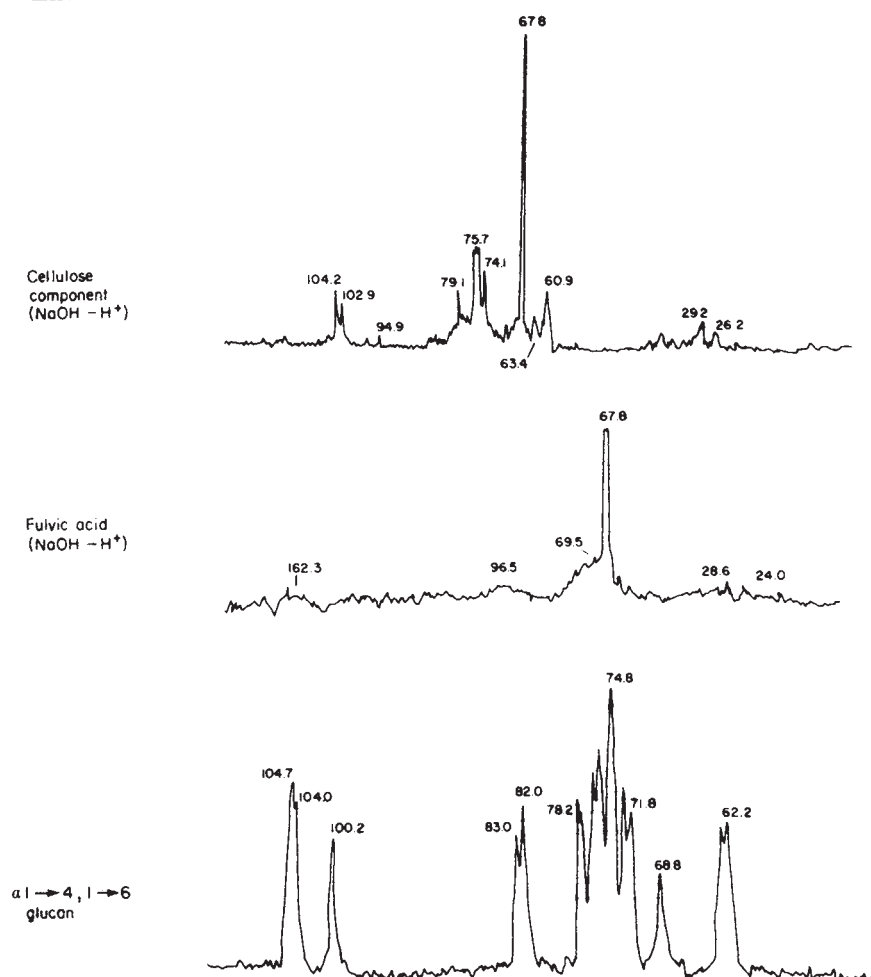


Fig. 3 ^{13}C -NMR spectra of a fulvic acid, a cellulose component and a glucan, internal standard = d_6 -dioxane (67.8 p.p.m.) all in $\text{NaOD}/\text{D}_2\text{O}$ (1M).

^{13}C -NMR absorptions that might be assigned to aromatic carbons.

A peak at 174.33 p.p.m. identified in our spectra agrees well with that reported elsewhere⁴ (174 p.p.m.); this can be attributed to carboxylic groups, esters and amides, but primarily to carboxylate entities⁴.

The $\text{Na}_4\text{P}_2\text{O}_7$ -extracted, acid-precipitated humic material (II) gave a ^{13}C -NMR spectrum with a very high background noise level and very broad peaks (Fig. 2). This can be attributed to the high ash content, which include paramagnetic ions, principally Fe. There is a corresponding loss of information, with only three main absorption bands, 44.5 p.p.m. (aliphatic), 106.5 p.p.m. (alkenic or aromatic) and 132.12 (alkenic or aromatic).

The humic acid III spectrum (Fig. 2) suffered from the same defect, but sharp split peaks appeared at 40–47 p.p.m. Six-membered aliphatic rings also absorb partially in this region.

A humic acid precursor, cellulose, showed a visible precipitate on acidification of a basic extract, the yield being ~100 mg/per 50 g acid-washed cellulose; yields would have been higher for unwashed cellulose. Sucrose, glucose or galacturonic acid gave no precipitates using the classical extraction scheme.

The similarity of the cellulose precipitate to that of humic and fulvic acid revealed by the IR spectra (Fig. 1) is consistent with cellulose being an integral part of the innate structure of humic substances as well as a precursor.

^{13}C -FT-NMR spectra revealed some similarity between the cellulose precipitate spectrum compared with the fulvic acid (Fig. 3) or humic acid IV (Fig. 2) spectra and hemicellulose component (Fig. 3)—an $\alpha 1 \rightarrow 4, 1 \rightarrow 6$ glucan³⁵ was reasonably similar to that of the cellulose precipitate (Fig. 3). NaOH (0.2 M) is an efficient extractant for polysaccharides from soil³⁶ and hence such hemicellulose components must be an integral part of the classical fractionation scheme.

Acid-base treatment of carbohydrates (glucose) yields aromatic compounds³⁷ even at pH 3.5, including dihydroxybenzenes and dihydroxybenzoic acids which are considered model monomers of humic substances. Although yields were low (3.5%) they may increase significantly in the presence of potential endogenous catalysts such as clays and transition metal cations. Solutions of carbohydrates in 0.63 M NaOH resulted in degradation to a large number of phenols including humic precursors³⁸.

The classical acid-base extraction scheme may artificially cause aromatic structures to form from indigenous carbohydrates. This would result in aromatic signals or absorptions in ^{13}C -NMR, ^1H NMR and IR spectra. Note that the ^{13}C -NMR spectrum for fulvic acid solution of polysaccharides in fulvic and humic acids extracted from sediments¹ (Fig. 1) is very similar to that shown for the hemicellulose component isolated in our studies (Fig. 3).

Our studies suggest that humic substances may not be as aromatic as previously thought, that the extraction technique is very important in determining aromaticity that paramagnetic ions can be critical in determining spectral features, and that

Table 1 Transition metal ion contents of humic and fulvic acids studied

Sample	Fe	Mn	Cu	Zn
HA-I	0.47%	22	1315	120
HA-II	2.76%	175	275	147
HA-III	1.96%	848	105	114
HA-I-IV	68	ND	114	20
FA-I	533	4	52	33

Contents measured in p.p.m. unless indicated. HA, humic acid; FA, fulvic acid.

hemicellulosic compounds may be essential components of fulvic acid and humic acid structures.

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- Hatcher, P. G., Berger, I. A. & Mattingly, M. A. *Nature* **285**, 560–562 (1980).
- Villa, F. J. G., Martin, F., Saiz-Jimenez, Lentz, H. & Ludemann, H. D. *Agrochimica* **22**, 501–506 (1978).
- Stuerner, D. H. & Payne, J. R. *Geochim. cosmochim. Acta* **46**, 1109–1114 (1976).
- Wilson, M. A. & Goh, K. M. *J. Soil Sci.* **28**, 645–652 (1977).
- Ruggiero, P., Interesse, F. S. & Sciacovelli, O. *Geochim. cosmochim. Acta* **43**, 1771–1775 (1979).
- Grant, D. *Nature* **270**, 709–710 (1977).
- Wilson, M. A., Jones, A. J. & Williamson, B. *Nature* **276**, 487–489 (1978).
- Swift, R. S. & Posner, A. M. *J. Soil Sci.* **23**, 381–393 (1972).
- Ceccanti, B., Nannipieri, P., Cervelli, S. & Sigui, P. *Soil Biochem.* **10**, 39, 1978.
- Kononova, M. M. & Alexandrova, I. V. *Geoderma* **9**, 157–164 (1973).
- Gieseking, J. E. *Soil Components* Vol. 1 (Springer, New York, 1975).
- Lenz, H., Ludemann, H. D. & Ziechmann, W. *Geoderma* **18**, 325–328 (1977).
- Sciacovelli, O., Gessa, C., Ruggiero, P. A. & Testini, C. *Soil Biol. Biochem.* **12**, 389–395 (1980).
- Brunow, G. & Lemmetyinen, R. *Acta Chem. Scand.* **B32**, 545–546 (1978).
- Hofe, G. *Tetrahedron* **32**, 1431–1436 (1976).
- Kusumi, T., Shibata, Y., Bhitsuka, M., Kinoshita, T. & Kakisawa, H. *Chem. Lett.; Chem. Soc. Jap.* 277–278 (1979).
- Visser, S. A. *J. Soil Sci.* **15**, 202–219 (1964).
- Given, P. H. & Dickenson, C. H. *Soil Biochem.* **3**, 123–211 (1974).
- Sauerbeck, D. & Fuhr, F. *Soil Organic Matter* No. 3–11 (IAEA, Vienna, 1968).
- Swift, R. S. & Posner, A. M. *Soil Organic Matter* No. 211/19, 171–182 (IAEA, Vienna, 1972).
- Zhigunov, A. V. & Simakov, V. N. *Pochvooverdenie* **12**, 59–65 (1977).
- Robertson, J. B. *Topics in Dietary Fiber Research* Ch. 1, 1–41 (Plenum, New York, 1978).
- Williams, K. T. & Bevenue, A. J. *Ass. Offic. analyt. Chem.* **39**, 901–918 (1956).
- Schultze, H. A. *Physiol. Chem.* **16**, 387 (1892).
- Shorey, E. C. & Martin, J. B. *J. Am. chem. Soc.* **52**, 4907–4915 (1930).
- Theng, B. K. G., Wake, J. R. H. & Posner, A. M. *J. Soil Sci.* **18**, 349 (1967).
- Dyer, J. R. *Applications of Absorption Spectroscopy of Organic Compounds* (Prentice Hall, New York, 1965).
- Bovey, F. A. *High Resolution NMR of Macromolecules* (Academic, New York, 1972).
- Abraham, R. J. & Loftus, P. *Proton, and C-13 NMR Spectroscopy—An Integrated Approach* (Heyden, London, 1978).
- Levy, G. C. & Nelson, G. L. *Carbon-13 NMR for Organic Chemists* (Wiley, New York, 1972).
- Sposito, G., Schaumberg, G. D., Perkins, T. G. & Holtzclaw, K. M. *Envir. Sci. Technol.* **12**, 931–934 (1978).
- Wilson, M. A. & Goh, K. M. *Plant Soil* **46**, 287–289 (1977).
- Villa, F. J. G., Lentz, H. & Ludemann, H. *Biochem. biophys. Res. Commun.* **72**, 1063–1070 (1975).
- Onner, G. *Geochim. cosmochim. Acta* **43**, 105–108 (1979).
- Jennings, H. J. & Smith, I. C. P. *J. Am. chem. Soc.* **95**, 606–608 (1973).
- Cheshire, M. V. *J. Soil Sci.* **28**, 1–10 (1977).
- Popoff, T. & Theander, O. *Acta Chem. Scand. Ser. B30*, 2–7 and 397–402 (1976).
- Forsskahl, I., Popoff, T. & Theander, O. *Carbohydr. Res.* **48**, 13–21 (1976).

Land plant evidence compatible with gradual, not catastrophic, change at the end of the Cretaceous

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Field study of the fossil and sedimentary record across the Cretaceous–Tertiary (K–T) boundary in Wyoming and Montana has been combined here with a reassessment of the published record of terrestrial palynomorphs to test recent hypotheses that a universal biotic catastrophe caused by an asteroid^{1–3}, cometary impact⁴ or a supernova⁵ terminated the Cretaceous. Evidence from land plants is particularly critical because plants form the base of the terrestrial food chain. Thus, massive disruptions of the Earth's vegetation by the postulated effects of a cosmic disaster (blocking of solar radiation for several years by a dust cloud¹, severe atmospheric heating^{1,4,6,7} or ionizing radiation⁵) would have an amplified effect on the land fauna. However, I report here that the geographically uneven and generally moderate levels of extinction and diversity change in the land flora, together with the non-synchronicity of plant and dinosaur extinctions, contradict hypotheses that a catastrophe caused terrestrial extinctions.

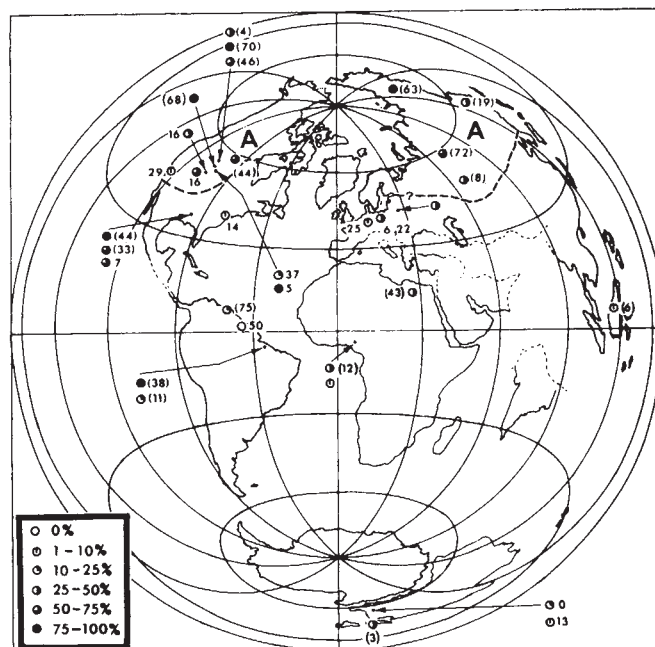


Fig. 1 Severity of plant extinction (mostly at the species level) and change in diversity across the Cretaceous–Tertiary boundary. The dashed line represents the approximate limits of the Aquilapollenites province (designated 'A') covering northwestern North America and northeastern Asia. The symbols for localities indicate the percentage of Cretaceous plants that became extinct across the K–T boundary, and are explained in the key. The figures accompanying the symbols indicate the percentage change in diversity with negative values indicated by parentheses. Levels of extinction >50% reported at two localities outside the Aquilapollenites province are attributable, in the case of the Mississippi Embayment, to facies changes⁴⁷ or restriction of the sample to the Normapolles group of pollen, which fared badly across the K–T boundary; and, in the case of northeastern Brazil, to a restricted sample of indicator species¹². (Base map for the early Palaeocene redrawn from ref. 48.)

Fossil plants, mainly palynofloras, provide a detailed picture of terrestrial change across the K–T boundary that has been sampled throughout much of the world. Unfortunately, this record does not yet offer the degree of time–stratigraphical resolution possible for the marine sequence because of the relatively few studies across the boundary, wide spacing of samples and frequent emphasis on recognition of the Cretaceous or of the Palaeocene using selected guide fossils rather than by characterizing the whole assemblage. In addition, shifts in sedimentation across the boundary are seldom documented but may strongly influence the floristic composition; megafossil remains have been extensively misidentified^{8–11} and serious problems of correlation exist between marine and non-marine stratigraphical sections as well as between sections on different continents. Despite these difficulties, a coherent pattern of moderate floristic change can be discerned across the K–T boundary¹².

To standardize the extinction figures reported here, all late Maastrichtian forms not found in the Lower Palaeocene were treated as though they became extinct at the K–T boundary. These extinction figures were also based on geographically restricted floras which probably record local range changes as well as extinctions. Both these factors elevate the apparent level of extinction.

Late Cretaceous floras were dominated by the flowering plants, which appeared in the mid–early Cretaceous^{6,13–17} and achieved dominance by the Turonian^{6,18} in the Northern Hemisphere but perhaps not until the Maastrichtian¹⁸ in the Southern Hemisphere. During the mid–late Cretaceous, the pollen flora of the middle- and high-latitude Northern

Hemisphere gradually diverged into two distinct provinces^{19,20}. The area from eastern North America across Europe into western Asia was dominated by the Normapolles group. A second province extended from western North America nearly across Siberia and was characterized by forms of unusual morphology, known as the Aquilapollenites group^{21,22}, along with, for example, *Wodehousia*, *Azonia* and *Proteacidites*. Both pollen and leaf floras were dominated by archaic taxa with modern forms such as the palms, *Nothofagus*^{18,20}, *Platanus*^{23,24} and a few others^{20,25}, appearing in small numbers gradually through the late Cretaceous.

At the end of the Cretaceous, the most noteworthy change in the world land flora was the decimation of the Aquilapollenites province. Within its boundaries, extinction often amounted to 75% of the Cretaceous species and showed an imperfect pattern of increase towards the north (Figs 1, 2). Elsewhere the level of extinction was much less, seldom exceeding 50% of Cretaceous forms¹². Floral diversity also decreased most markedly in the Aquilapollenites province with the level of diversity attenuation generally increasing northwards. Megafossil studies across the K-T boundary are limited to western North America but show similar levels of extinction of Cretaceous forms (40–60%)¹² and a substantial increase in the proportion of toothed (that is, cooler climate^{26,27}) leaves.

For comparison, the same method of analysis was applied to extinction across the Palaeocene–Eocene boundary, where no catastrophe has ever been invoked. In North Dakota a third of the palynoflora²⁸ and one-half of the megafloora²⁹ became extinct through a section starting 10 m below the boundary. Similar levels of extinction are seen by comparing the total Clarkforkian (latest Palaeocene) with the total Wasatchian (early Eocene) megafloora of the Bighorn Basin in Wyoming (S. L. Wing, thesis in preparation, Yale University).

Given a terminal Cretaceous event of several to several hundred years duration and of overwhelming magnitude, extinctions in various terrestrial groups should appear synchronous at our resolution limits. Only in western North America have stratigraphical sections containing both the last dinosaurs and plants been carefully observed. There, the Cretaceous flora persists above the level of the highest unreworked dinosaur bone. In Alberta the floral change occurs 6 m higher³⁰. In east central Montana³¹, northwestern Wyoming (my observations and P. Gingerich, personal communication) and southeastern Wyoming³², the change occurs at a minimum of 2–3 m higher. In Colorado the change lies an unspecified distance above the last dinosaur³³. Based on a sedimentation rate of 65 m Myr⁻¹ for the Edmonton Group³⁴, the stratigraphical interval between the last dinosaur and the floral change would represent ~50–90 × 10³ yr. In addition, several authors note an attenuation in dinosaur

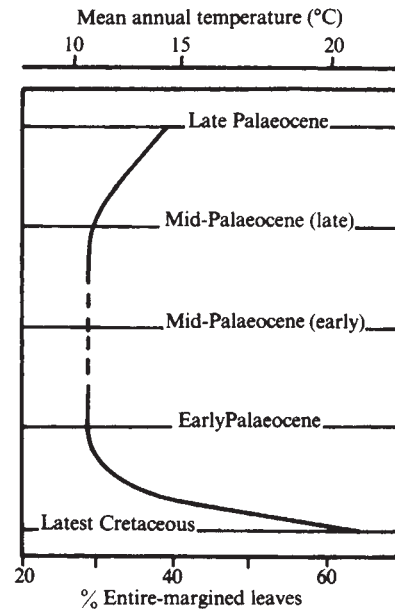


Fig. 3 Changes in the percentage of entire-margined, dicotyledonous angiosperm leaves and inferred palaeotemperature changes from the latest Cretaceous through the Palaeocene in the Bighorn Basin of northwestern Wyoming (modified from ref. 72).

abundance and diversity towards the boundary^{31,34}, a finding corroborated by my field work in north-west Wyoming.

Generally moderate, geographically variable levels of land-plant extinction and the lack of synchronicity in plant–dinosaur extinctions at the close of the Cretaceous make it unlikely that a universal biotic catastrophe occurred. Despite a lower thermal gradient in the Cretaceous^{35,36} due to lack of polar ice caps³⁶ and a generally higher average Earth temperature³⁷, Northern Hemisphere floras show an increase of cooler-temperature forms with increasing latitude³⁷, though more gradually than at present. This suggests a correlative increase in the efficiency of plant dormancy mechanisms as well as chromosomal redundancy, both of which would have raised resistance to environmental stress^{38–40}. The elevated levels of extinction seen at higher northern latitudes across the K–T boundary effectively contradict the assertion that the Earth's vegetation was simply able to regenerate after cosmic catastrophe^{4,5}. In addition, low-latitude floras, relatively lacking in such dormancy and carryover mechanisms, suffered least, exactly the opposite of that expected from the catastrophic model (Figs 1, 2).

Alternatively, the pattern of change in land plants, the increasingly cooler affinities of latest Cretaceous to early Palaeocene palynofloras^{36,41–44}, the increase in the percentage of forms with toothed leaves in the western United States¹², the attenuation of dinosaurs and the lack of synchronicity in extinction of plants and dinosaurs are compatible with climatic cooling. A palaeotemperature curve for Montana and northern Wyoming suggested by megafossil evidence (Fig. 3) and numerous marine palaeotemperature curves point to climatic cooling across the boundary with gradual recovery in the Palaeocene. (Recent suggestions of a very short-term temperature increase at the boundary^{6,7,45,46} cannot be seen at the time scale of the floral studies reviewed here.) An attenuated dust cloud, rather than the completely opaque one of Alvarez *et al.*, would have caused relatively less light to reach high latitudes due to the greater screening power of dust at the increasing angles of insolation that occur there. The result would have been a steepened gradient of declining temperatures towards high latitudes and an attendant increase in plant extinctions. However, the effect of such a dust cloud would have been of very short duration. Furthermore, this hypothesis fails to account for the lack of appreciable extinction or diversity decline at high

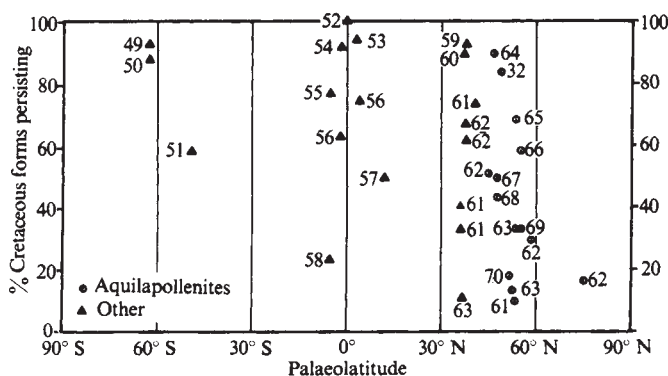


Fig. 2 Survival of Cretaceous plant species across the K–T boundary as a function of palaeolatitude. Localities falling in the Aquilapollenites province have been distinguished from those in other regions. Reference numbers appear beside the points. Note the generally high levels of floral survival from high-southern to mid-northern palaeolatitudes.

southern latitudes (Figs 1, 2) or the irregular occurrence of such effects elsewhere. These are more satisfactorily explained by long-term climatic deterioration, whose influence would be far less uniform over the Earth's surface than that of a dust cloud.

The asteroid hypothesis has stimulated discussion between physical scientists and palaeontologists. However persuasive and well documented the evidence for an iridium anomaly, the connection between it and the terminal Cretaceous extinctions is tenuous at best. Before a general theory explaining these extinctions can be framed, additional data on geochemical causes of the Ir anomaly, the range of physical consequences of a postulated cosmic catastrophe, and the timing and magnitude of late Cretaceous extinctions must be gathered and evaluated.

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1. Alvarez, L. W. *et al.* *Science* **208**, 1095–1108 (1980).
2. Smit, J. & Hertogen, J. *Nature* **285**, 198–200 (1980).
3. Gagnapathy, R. *Science* **209**, 921–923 (1980).
4. Hsu, K. J. *Nature* **285**, 201–203 (1980).
5. Russell, D. & Tucker, W. H. *Nature* **229**, 553–554 (1971).
6. Hughes, N. F. *Palaeobiology of Angiosperm Origins* (Cambridge University Press, 1976).
7. McLean, D. M. *Science* **201**, 401–406 (1978).
8. Pacltová, B. *Prestia* **33**, 113–129 (1961).
9. Wolfe, J. A. *Am. J. Bot.* **59**, 664 (1972).
10. Hickey, L. J. *Am. J. Bot.* **60**, 17–33 (1973).
11. Dilcher, D. L. *Bot. Rev.* **40**, 1–1547 (1974).
12. Hickey, L. J. in *Catastrophes in Earth History: The New Uniformitarianism* (eds Berggren, W. A. & Van Couvering, J. A.) (Princeton University Press, in the press).
13. Doyle, J. A. & Hickey, L. J. in *Origin and Early Evolution of Angiosperms* (ed. Beck, C. B.) 139–206 (Columbia University Press, New York, 1976).
14. Hickey, L. J. & Doyle, J. A. *Bot. Rev.* **43**, 3–104 (1977).
15. Hughes, N. F. *Bot. Rev.* **43**, 3–104, 105–127 (1977).
16. Vakhrameev, V. A. & Kotova, I. Z. *Paleont. Zh.* **1977**, 101–109 (1977).
17. Doyle, J. A. *et al.* *Bull. Ant. Rech. Explor.-Prod. Elf-Aquitaine* **1**, 451–473 (1977).
18. Penny, J. S. in *Aspects of Palynology* (eds Tschudy, R. H. & Scott, R. A.) 331–376 (Wiley-Interscience, New York, 1969).
19. Doyle, J. A. *J. Arnold Arb.* **50**, 1–35 (1969).
20. Muller, J. *Biol. Rev.* **45**, 417–450 (1970).
21. Mchedlishvili, N. D. *Trudy VNIGRI* **177**, 1–342 (1961).
22. Stanley, E. A. *Bull. Ga Acad. Sci.* **28**, 1–44 (1970).
23. Krassilov, V. A. *Palaeontographica* **B142**, 105–116 (1973).
24. Pacltová, B. *Cour. Forsch.-Inst. Senckenberg* **30**, 70–76 (1978).
25. Niklas, K. J., Tiffney, B. H. & Knoll, A. H. in *Evolutionary Biology* (eds Hecht, M. K., Steere, W. C. & Wallace, B.) 1–89 (Plenum, New York, 1980).
26. Bailey, I. W. & Sinnott, E. W. *Science* **41**, 832–833 (1915).
27. Wolfe, J. A. *Prof. Pap. U.S. geol. Surv.* **1106**, 34–35 (1979).
28. Hickey, L. J. *Mem. geol. Soc. Am.* **150**, 57–71 (1977).
29. Bebout, J. W. thesis, Pennsylvania State Univ. (1977).
30. Lerbekmo, J. F., Evans, M. E. & Baadsgaard, H. *Nature* **279**, 26–30 (1979).
31. Clemens, W. & Archibald, D. *Mém. Soc. géol. Fr.* **139**, 67–74 (1980).
32. Leffingwell, H. A. *Spec. Pap. geol. Soc. Am.* **127**, 1–64 (1970).
33. Newman, K. B. in *Cretaceous-Tertiary Boundary Events II* (eds Christensen, W. K. & Birkelund, T.) 246–248 (University of Copenhagen, 1979).
34. Van Valen, L. & Sloan, R. E. *Evol. Theory* **2**, 37–64 (1977).
35. Gartner, S. in *Cretaceous-Tertiary Boundary Events II* (eds Christensen, W. K. & Birkelund, T.) 26–28 (University of Copenhagen, 1979).
36. Saito, T. & Van Dong, J. *Micropaleontology* **20**, 152–177 (1974).
37. Savin, S. M. A. *Rev. Earth planet. Sci.* **5**, 319–355 (1977).
38. Raunkiaer, C. *The Life Forms of Plants and Statistical Plant Geography: Being the Collected Papers of C. Raunkiaer* (Clarendon Press, Oxford, 1934).
39. Cain, S. A. *Bot. Rev.* **16**, 1–32 (1950).
40. Woodwell, G. M. *Scient. Am.* **208** (6), 40–49 (1963).
41. Samilovich, S. R. *Rev. Palaeobot. Palynol.* **2**, 127–139 (1967).
42. Smiley, C. J. *Geosci. Man* **4**, 91–99 (1972).
43. Srivastava, S. K. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **7**, 221–276 (1970).
44. Krassilov, V. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **17**, 152–172 (1975).
45. Boersma, A. *et al.* *Init. Rep. DSDP* **43**, 695–719 (1979).
46. Boersma, A. & Shackleton, N. in *Cretaceous-Tertiary Boundary Events II* (eds Christensen, W. K. & Birkelund, T.) 50–53 (University of Copenhagen, 1979).
47. Jarzen, D. M. *Pollen Spores* **20**, 535–553 (1978).
48. Smith, A. G. & Birden, J. C. *Mesozoic and Cenozoic Paleogeographic Maps* (Cambridge University Press, 1977).
49. Stover, L. E. & Partridge, A. O. *Proc. R. Soc. Vict.* **85**, 237–286 (1973).
50. Stover, L. E. & Evans, P. R. *Spec. Publ. geol. Soc. Aust.* **4**, 55–72 (1973).
51. Couper, R. A. *Palaeont. Bull., Wellington* **32**, 1–84 (1960).
52. Leidelmeyer, P. *Leid. geol. Meded.* **38**, 49–70 (1966).
53. Muller, J. *Micropaleontology* **14**, 1–37 (1968).
54. van Hocken-Klinkenberg, P. M. J. *Leid. geol. Meded.* **38**, 37–48 (1966).
55. Müller, H. in *Proc. of the Second West African Micropaleontological Colloquium*, Ibadan, No 5 (ed. van Hinte, J. E.) 123–136 (Brill, Leiden, 1966).
56. Germeraad, J. H., Hopping, C. A. & Muller, J. *Rev. Palaeobot. Palynol.* **6**, 189–348 (1968).
57. Kedevs, M. *Acta bot. sci. Hung.* **17**, 371–378 (1971).
58. de Boer, N. P., van der Hammen, T. & Wymstra, T. A. *Geologie Mijnb.* **44**, 254–258 (1965).
59. Krusch, W. Z. *Angew. Geol.* **3**, 509–548 (1957).
60. Zaklinskaya, E. D. in *Razvitiye Flor na Granitne Mezozoya i Kainozoya* (ed. Vakhrameev, V. A.) 66–119 (Nauka, Moscow, 1977).
61. Tschudy, R. H. *Prof. Pap. U.S. geol. Surv.* **865**, 1–42 (1975).
62. Zaklinskaya, E. D. in *Paleozoiskie i Mezozoiskie Flori Evrazii i Fitogeografiya etogo Vremeni* (eds Vakhrameev, V. A. *et al.*) 302–331 (Nauka, Moscow, 1970).
63. Tschudy, R. H. *Spec. Pap. geol. Soc. Am.* **127**, 65–111 (1970).
64. Drugg, W. S. *Palaeontographica* **B120**, 1–71 (1967).
65. Shoemaker, R. E. *Palaeontographica* **B119**, 54–75 (1966).
66. Mamontova, I. V. in *Paleobotanika na Dal'nem Vostoke* (ed. Krassilov, V. A.) 32–37 (Institute of Biology and Pedology, Vladivostok, 1977).
67. Dorf, E. *Bull. geol. Soc. Am.* **51**, 213–236 (1940); *Publs Carnegie Instn* **508**, 79–159 (1942).
68. Dorf, E. *Publs Carnegie Instn* **508**, 1–78 (1938).
69. Vakhrameev, V. A. & Achmetev, M. A. in *Razvitiye Flor na granitne Mezozoya i Kainozoya* (ed. Vakhrameev, V. A.) 39–65 (Nauka, Moscow, 1977).
70. Stanley, E. A. *Bull. Am. Paleont.* **49**, 179–383 (1965).
71. Norton, N. J. & Hall, J. W. *Palaeontographica* **B125**, 1–92 (1969).
72. Hickey, L. J. *Contr. Mus. Paleont. Univ. Mich.* **24**, 33–49 (1980).

Spherical phosphatic microfossils from the Silurian of North Greenland

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We report here that during routine micropalaeontological processing of Silurian limestone samples from North Greenland, a large number of small spherical, spinose microfossils have been isolated. These are comparable with forms studied in thin section by Sannemann¹, which have been referred to the group *Acritarcha*, erected by Evitt² to accommodate hollow, organic-walled microfossils whose affinities are uncertain. Our material suggests that the walls of the spherical bodies were mineralized rather than organic, throwing doubt on an assignment to the *Acritarcha*.

The specimens we have examined are from the upper part of the unnamed Silurian limestone formation and the unnamed Silurian black shale formation³ of Central Peary Land and their equivalents in Washington Land⁴, North Greenland. The samples were collected by field geologists of the Geological Survey of Greenland and processed in dilute acetic acid at Nottingham to recover conodonts, other phosphatic microfossils and organic microfossils. Five samples (GU sample nos 216849, 216853, 228978, 229036 and 228926) have yielded a total of more than 3,000 microspheres; associated conodonts include *Pterospiriferus celloni* (Walliser) and indicate a late Llandovery age⁵. Samples processed using standard palynological techniques (involving the use of hydrochloric and hydrofluoric acids) for the recovery of organic-walled microfossils have failed to yield any similar bodies.

The size of the microspheres varies from 100 to 400 µm in diameter, and long spines, which are broken on almost all specimens, project from the surface (Fig. 1). The density of these spines is variable and their basal insertion ranges from parallel-sided on some specimens (Fig. 1a) to broadly flaring in others (Fig. 1b). The central body may be smooth (Fig. 1a), granular (Fig. 1e, h) or covered with pillars up to 10 µm long and 2 µm in diameter (Fig. 1f, i). Scanning electron microscope examination of broken specimens has revealed several with complex wall structures (Fig. 2b–d). Commonly, an outer, thin, single layer is separated by 5–17 µm from a double inner layer by stout pillars. On one specimen (no. MGUH 15322) we have observed a third inner, structureless layer (Figs 2b, c, 3b). Each wall layer except the latter is composed of crystallites arranged perpendicular to the surface (Fig. 2a, c) and is less than 1 µm thick. The spines arise from the inner layer (Fig. 2d) and also commonly display a double wall; the lower portions of the spines seem to be hollow (Fig. 2a, d). Although some of our specimens show simpler wall structures, this may be the result of removal or non-fossilization of the outer layers.

Energy dispersive analysis of the spheres showed high calcium (Fig. 3c, d) and high phosphorus (Fig. 3e, f) content of the outer surface and of the inorganic wall layers, indicating that they are composed of apatite. Repeated 20-s counts of the PKα and CaKα peaks on two microsphere specimens provided Ca:P ratios of 1.99 and 2.12, respectively. Two apatite standards

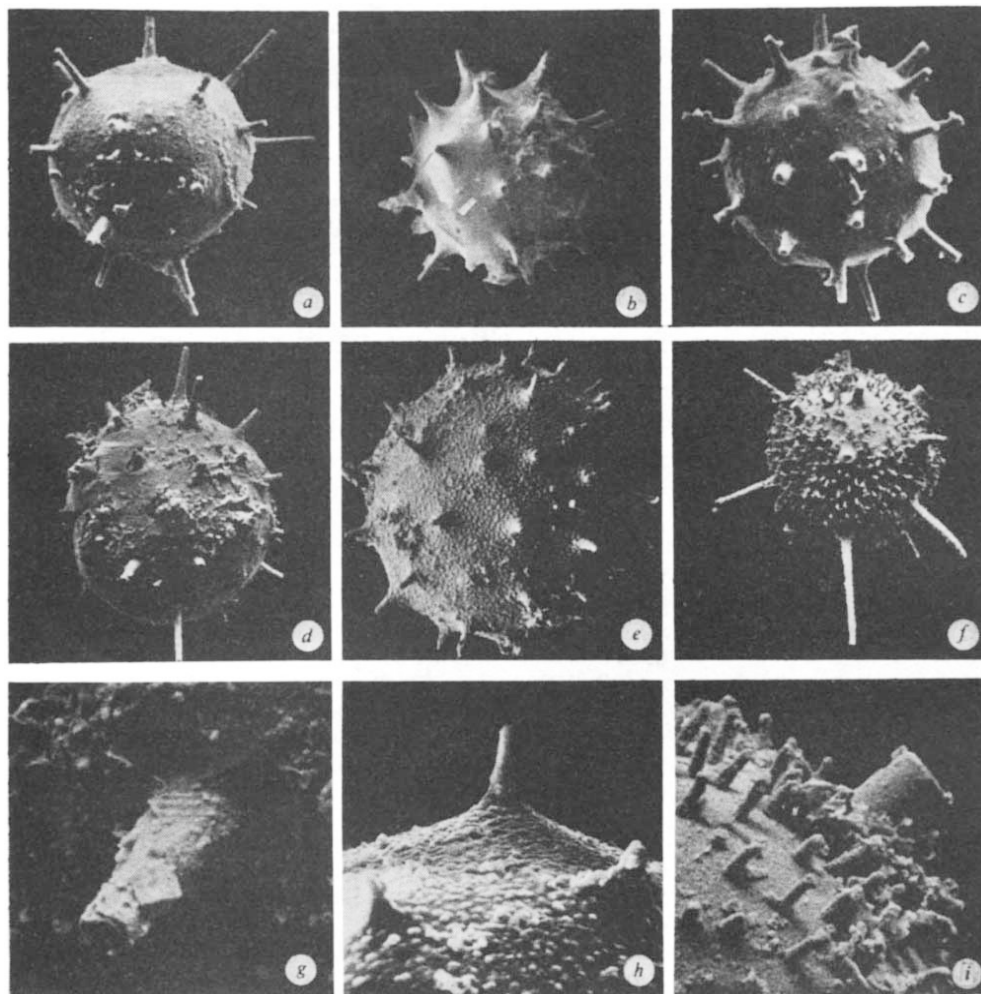


Fig. 1 Variation in external morphology of the microspheres. *a*, Specimen MGUH 15315, $\times 138$. *b*, Specimen MGUH 15316, $\times 165$. *c*, Specimen MGUH 15317, $\times 225$. *d*, Specimen MGUH 15318, $\times 150$. *e*, Specimen MGUH 15319, $\times 213$. *f*, Specimen MGUH 15320, $\times 130$. *g*, Specimen MGUH 15318, $\times 1368$, showing spine insertion and partial breakdown of outer layer round spine. *h*, Specimen MGUH 15319, $\times 645$. *i*, Specimen MGUH 15320, $\times 515$. The pillared and granular 'ornament' on these specimens may represent the remains of the pillar supports of the outer wall, which has not been preserved. All specimens are from GGU sample 216849 and numbers relate to the collections of the Geologisk Museum, Copenhagen.

analysed at the same time yielded ratios of 1.88 and 2.10 and a coeval conodont specimen, composed of organically secreted apatite, showed a ratio of 2.19. Published analyses of apatite are mostly in the same range^{6,7}, although our specimens seem to fall outside the range of francolite (Ca:P 2.5–2.7), a common diagenetic carbonate apatite in sedimentary environments⁸. The third inner layer of specimen MGUH 15322 produced no peaks on energy dispersive analysis, which is sensitive to elements with an atomic number of ≥ 11 . It is probable that this layer is organic in composition.

In assessing the biological affinities of the microspheres it is important to determine that the phosphatic mineralization of the walls is primary, and not a result of replacement during fossilization. Unfortunately, there is no unequivocal criterion. Circumstantial evidence is provided by the fact that there is very little phosphate in our limestone samples and that calcareous crinoid and brachiopod fragments show no signs of phosphatization. A chitinozoan (with an organic wall of 'pseudo-chitin'), from the sample with the most abundant microspheres, shows minor silicification and no trace of calcium phosphate. Hence, if the phosphatization is secondary, it has been extremely selective and mineralogically unusual. The phosphatic wall structure of the spheres is comparable with that of other phosphate-secreting organisms, such as lingulid brachiopods⁹, and it is more probable that the apatite is primary. However, some features of surface sculpture may be the result of diagenetic effects.

The shape of the microspheres is very similar to that of acanthomorphic acritarchs, which are normally smaller (average diameter 30 μm) and have organic walls. Sannemann¹ assigned his specimens from the Devonian of the Frankenwald, West Germany, to the genus *Hystrichosphaeridium* on the basis of the general similarity in morphology. Subsequently, they were

transferred to the genus *Baltisphaeridium*^{10,11} and were catalogued as acritarchs by Eisenack¹². He noted, however, that they were unusually large and seemed to be constructed differently from the 'true' acritarchs. These structural differences are emphasized by our material and the phosphatic walls firmly rule

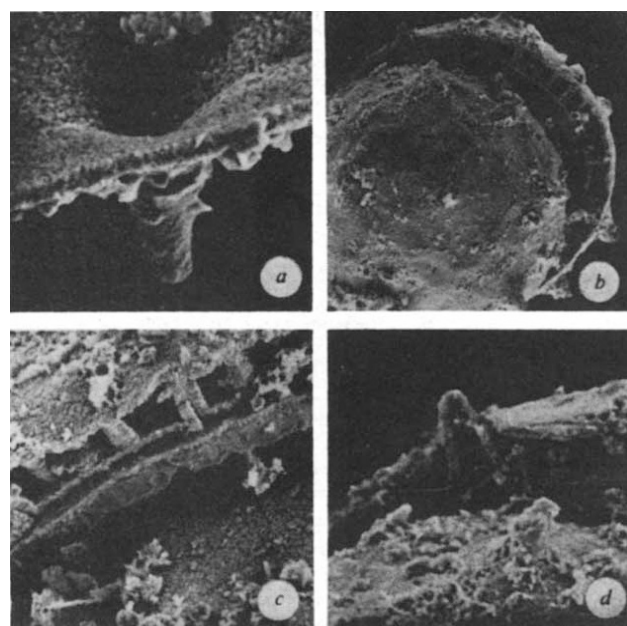


Fig. 2 Wall ultrastructure of the microspheres. *a*, Specimen MGUH 15321, $\times 800$. *b–d*, Specimen MGUH 15322, $\times 215$, $\times 860$, $\times 1075$. Both specimens from GGU sample 216849.

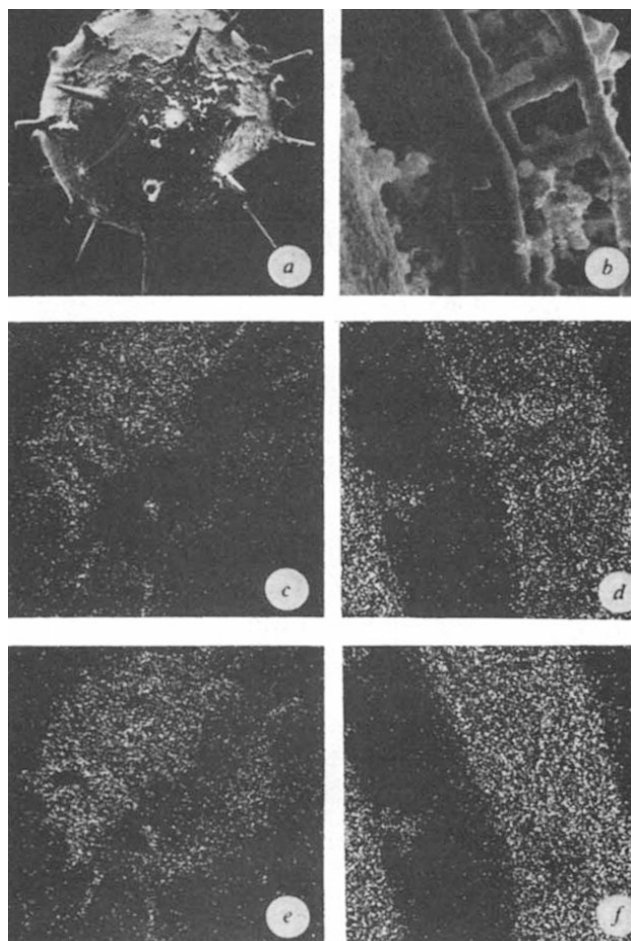


Fig. 3 Energy dispersive analysis of microsphere walls. *a*, Specimen MGUH 15323, $\times 270$. *b*, Specimen MGUH 15322, $\times 860$. *c*, *d*, Calcium distribution across the specimens in *a*, *b*. *e*, *f*, Phosphorus distribution across the specimens in *a*, *b*. Both specimens from GGU sample 216849.

out classification with the acritarchs. Spinose, spherical skeletons are also common among the Radiolaria¹³, but their walls are latticed, spongy or perforate, and we know of none that compare closely in structure with the microspheres. Modern radiolarians have skeletons of opaline silica, although this may be replaced by other minerals in fossil material.

Somewhat similar structures are shown by microgranular foraminifera referred to the superfamily Parathuramminacea¹⁴, especially the genera *Parathuramina*, *Archaeosphaera* and *Uralinella*. These, however, lack the perfect sphericity and complexity of wall structure shown by the microspheres, and preservation in phosphate would demand replacement of the original calcium carbonate tests. Finally, there is some similarity between our microspheres and various spherical calcium carbonate microfossils that have been termed calcispheres¹⁵, particularly those referred to the radiosphaerid calcispheres¹⁶. The structure of radiosphaerid calcispheres is rather different, as they are characterized by a spinose or prismatic outer wall layer, and again, preservation in phosphate would demand diagenetic replacement. However, the calcispheres are a heterogeneous group of uncertain affinities, and it is possible that specimens comparable to the Greenland microspheres have, at some time, been assigned to that group. Even so, there is no known group that readily accommodates our specimens. Their spherical structure, multiple concentric wall layers and prominent spines are characteristic, and their probable original apatite composition sets them apart from other microfossils. For ease of reference, we informally name them mazuelloids; the name is derived from the mediaeval latin term for 'mace'.

We report the results of our preliminary studies of these microspheres in the hope that they will stimulate finds of more material. The mazuelloids are probably widely distributed, but commonly missed in micropalaeontological investigations. They would not be preserved in palynological residues as they collapse in hydrochloric and hydrofluoric acids, and their small size and delicacy may lead to them being overlooked or broken in routine microfossil searches. Indeed, since recovery of the Greenland material, we have isolated several additional phosphatic mazuelloids from the upper Silurian of Czechoslovakia.

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1. Sannemann, D. *Senckenberg. leth.* **36**, 321–346 (1955).
2. Eviitt, W. R. *Proc. natn. Acad. Sci. U.S.A.* **49**, 298–302 (1963).
3. Christie, R. L. & Peel, J. S. *Rapp. Grønlands geol. Unders.* **82**, 1–48 (1977).
4. Hurst, J. M. *Bull. Grønlands geol. Unders.* **138**, 1–95 (1980).
5. Aldridge, R. J. *Rapp. Grønlands geol. Unders.* **91**, 7–23 (1979).
6. McClellan, G. H. *J. geol. Soc. Lond.* **137**, 675–681 (1980).
7. Deer, W. A., Howie, R. A. & Zussman, J. *An Introduction to the Rock-Forming Minerals* (Longman, Oxford, 1974).
8. McArthur, J. M., Coleman, M. L. & Bremner, J. M. *J. geol. Soc. Lond.* **137**, 669–673 (1980).
9. Hewitt, R. A. *J. geol. Soc. Lond.* **137**, 661–667 (1980).
10. Eisenack, A. *Neues Jb. Geol. Paläont. Abh.* **106**, 383–422 (1958).
11. Downie, C. & Sarjeant, W. A. S. *Paleontology* **6**, 83–96 (1963).
12. Eisenack, A. *Katalog der fossilen Dinoflagellaten, Hystrichosphären und verwandten Microfossilien* Vol. 3, Pt. 1 (Schweizerbart, Stuttgart, 1973).
13. Kling, S. A. in *Introduction to Marine Micropalaeontology* (eds Haq, B. U. & Boersma, A.) 203–244 (Elsevier, New York, 1978).
14. Loeblich, A. R. & Tappan, H. in *Treatise on Invertebrate Palaeontology C* (ed. Moore, R. C.) (University of Kansas Press and the Geological Society of America, 1964).
15. Kązmierzak, J. *Acta palaeont. polon.* **21**, 245–255 (1976).
16. Stanton, R. J. Jr *Micropalaeontology* **13**, 465–472 (1967).

ESR-dating of the fossil hominid cranium from Petralona Cave, Greece

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The age of the hominid cranium discovered in 1960 in a limestone cave near Petralona (Greece) is a continuing cause of controversy. The age of the skull, which was apparently encrusted by brown calcite soon after the death of the individual concerned, has been variously estimated at between 70,000¹ and 700,000² yr. Here we show using electron spin resonance (ESR) measurements of the calcite encrustation and of bone fragments that the age of the Petralona hominid lies between 160,000 and 240,000 yr. We also demonstrate, by trace element analysis, that the composition of the calcite encrustation is the same as that of the very top of the travertine floor.

Part of the difficulty in interpreting the Petralona skull stems from the circumstance of its discovery, on the floor of the cave. The cave fauna has been variously attributed to the Riss/Würm³ and Günz/Mindel⁴ interstadial, suggesting that the hominid might be classified as *Homo sapiens neanderthaliensis* or *Homo erectus*. It is, however, questionable, whether faunistic remains

can be related to the hominid cranium. Previous estimates of the age have been based on measurements of speleothem samples either from other branches of the cave or from deeper (and thus older) strata. We believe that only the skull itself and its encrustation should be the basis for absolute age determination.

After inspecting the calcite encrustation, which was removed from the well preserved skull by J. W. Melentis (University of Saloniki, Greece) and one of us (N.I.X.), our attention was directed to some larger speleothem samples that were collected a few years ago in the cave near where the skull was discovered. As expected, some of these travertine samples were covered by a thin, opaque, reddish-brown, non-laminated calcite layer resembling the coating on the skull. The lack of any lamination indicates only one uninterrupted depositional event. To confirm the assumption of an identical growth horizon, pieces of the skull encrustation (a) and of the calcite layer (b) covering some of the larger speleothem fragments were analysed for several trace elements by instrumental neutron activation analysis (INAA). For comparison, a calcite sample (e) from 30–40 mm beneath the surface layer of the travertine floor was included.

Comparison of the results in Table 1 clearly shows the high similarity of samples (a) and (b). Sample (e) is quite different.

The higher K-, Mn- and Na-contents of sample (b) compared with (a) can be explained by an increased fraction of clay minerals. The similarity of (a) and (b) with respect to most of the analysed elements implies that both belong to the same layer of deposition⁵. This result is significant because practically all previous age determinations^{6–9} were done on samples from deeper strata (as, for example, sample (e)), but not from the thin brown top layer (b) of the travertine floor. These earlier works on Petralona speleothems attempted to correlate the massive base calcite stratum of the floor with the skull itself. However, a reliable stratigraphy with respect to the cranium has never been established. Consequently, a definite dating of this hominid was not possible.

For the present study, five different samples with clear stratigraphical relations to the hominid skull were chosen:

(a) Calcite encrusting the hominid skull, which should yield a lower age limit for the hominid.

(b) Top calcite layer covering the floor.

(c) Bone fragments of the hominid skull, which had accidentally come loose with the calcite encrustation (a).

(d) Calcite 3–4 mm beneath the surface layer (b), which should yield an upper limit for the hominid.

(e) Calcite 30–40 mm beneath the surface layer (b).

We considered that it was essential not to investigate only the small bone fragments of cranium (c), because although dating of animal and human bones by ESR has been reported¹⁰, bones may suffer from recrystallization and the hydroxylapatite can partially convert into fluorine-apatite. Calcite, however, is generally accepted as a stable mineral and a 'closed system'.

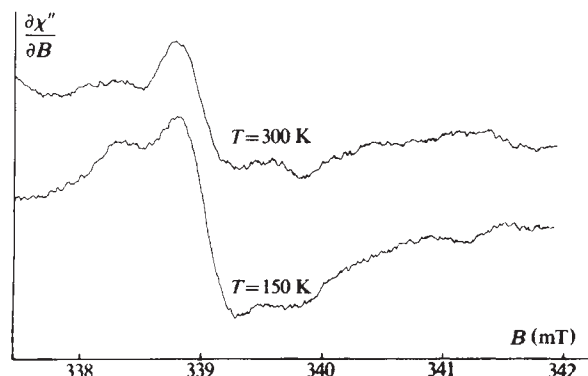


Fig. 1 ESR-spectra of bone fragments (c) of the Petralona hominid cranium, taken at 300 K and 150 K. $\nu = 9.5$ GHz, $p = 3.7$ mW, $m = 50$ mg.

Table 1 Trace element contents of calcite samples (a), (b) and (e), related to the hominid skull from Petralona Cave

Element	(a) (p.p.m.)	(b) (p.p.m.)	(e) (p.p.m.)
As	0.5	2.8	7.9
Co	1.7	1.3	0.02
Cr	13.3	14.3	0.32
Cs	1.9	1.1	0.01
Eu	0.09	0.08	0.004
Fe	4,700	4,600	140
K	87.4	377	≤ 10
Mn	42.3	163	0.44
Na	30.3	164	18
Rb	10.3	9.6	≤ 1
Sb	0.93	0.65	0.05
Sc	1.17	1.01	0.05
Th	1.84	1.39	≤ 0.1

Trace element concentrations determined by instrumental neutron activation analysis (INAA). There were two successive neutron irradiations of 3 min and 12 h: thermal neutron flux was 8×10^{13} neutrons $\text{cm}^{-2} \text{s}^{-1}$. Sample weights were ~ 150 mg, and average errors $\pm 10\%$.

Of the modern techniques such as thermoluminescence (TL)^{11,12}, electron spin resonance (ESR)^{13–15} and uranium series dating^{16–18}, which are suitable for dating Quaternary calcites, ESR seemed to be the most advantageous for the following reasons: (1) The presumed ages of all samples are beyond the limits of radiocarbon dating. (2) Only milligramme amounts of most samples were available, so that the low U content (< 1 p.p.m.) precludes the use of $^{230}\text{Th}/^{234}\text{U}$ -dating. (3) The high opacity of the bone and of some calcite samples is disadvantageous for TL-dating. The presence of a small clay fraction (of the order of a few parts per thousand) would cause strong interference with the TL-signal¹¹. The principle of ESR-dating has been described in detail elsewhere^{10,13–15}. In brief, ESR measures the concentration of paramagnetic electrons in defects of the crystal lattice, created by interactions with the natural radiation since the time of (speleothem) deposition. In the present study, ESR measurements were performed by means of a 'Bruker' spectrometer (BER 420) at a microwave frequency of 9 GHz (X-band) and a typical microwave power of 2 mW. The weighed samples were positioned in a variable, homogeneous magnetic field of ~ 300 mT.

For ESR-dating, ~ 1 g of each sample was cleaned from the adherent clay or bone impurities, ground, etched for 10 min in 0.1 M HCl and separated from the fine-grain fraction by decanting off the supernatant, milky solution. Portions of identical weight (generally 50–150 mg) were exposed to different γ -doses between 10 and 100 krad (1 krad = 10 J kg^{-1}) using a calibrated 6,000 Ci (1 Ci = $3.7 \times 10^{10} \text{ s}^{-1}$) ^{60}Co γ -source. One portion of each set of samples was left unirradiated to obtain the ESR-signal of the natural radiation dose, that is the 'archaeological' dose. The γ -irradiated samples were either left to stand for at least 1 day or were kept at 100°C for 1 h to remove the interfering (unstable) ESR signal, which presumably corresponds to the $\sim 80^\circ\text{C}$ TL glow peak.

The specific ESR-signal (proportional to the radiation dose) was sometimes subject to interference from other resonances which did not increase linearly with γ -doses (that is, it showed strong saturation effects). This problem was overcome by lowering the temperature of the sample to ~ 150 K using a helium gas-flow cryostat. At this temperature, not only was there better discrimination, but also a significant enlargement of the radiation-induced ESR signal. This signal enhancement is shown in Fig. 1 for the bone fragments (c).

To ensure that the radiation-induced ESR-signal (used for dating) had been stable for at least some 10^6 yr, annealing experiments were carried out. The initial disappearance of the ESR signal between 200°C and 250°C indicates a correlation with the 230°C TL glow peak of natural calcite. This TL signal, which shifts up to $\sim 275^\circ\text{C}$ when using a fast heating rate, has already been studied by Wintle¹⁹. Isothermal annealing experiments performed on ESR active defects chosen for dating

resulted in exponential signal decays with time constants τ according to an Arrhenius law $\tau = \tau_0 \exp(E_a/kT)$ with a pre-exponential $\tau_0 = 3.8 \times 10^{-14}$ s and a trap depth $E_a = 1.65 \pm 0.1$ eV. This allows us to deduce mean defect annealing times of $\tau = 9 \times 10^7$ yr and 3×10^8 yr for storage temperatures of 15 °C and 10 °C, respectively. These data are in good agreement with the mean lives given by Wintle for TL-dating¹⁹. Thus speleothem dating should be possible using ESR techniques up to a few million years without significant corrections for annealing, particularly as no anomalous fading is observed in the TL of calcite¹².

The heights of the radiation-induced ESR signals were recorded as well as one of the six absorption lines of Mn^{2+} , which are present in practically all ESR spectra of natural calcites. It has been suggested¹⁵ that these lines be used instead of sample weight for the calibration of the radiation-induced ESR-signals. Where possible, we applied both methods of calibration to evaluate the γ -equivalent archaeological doses (EDs).

In the same way the γ -equivalent archaeological doses (EDs) were determined for the other four samples (b)–(e). Results are listed in Table 3.

To obtain the formation ages in years, the EDs are divided by the corresponding radiation doses per year. To determine these annual doses requires that for each sample (see Table 2): (1) the U, Th, K and Rb contents have to be analysed; (2) possible disequilibria in the natural decay series have to be considered; (3) the efficiency of α -radiation (relative to an identical γ -dose) with regard to the radiation-induced ESR signal has to be determined; (4) the external γ -dose rates at the original sites of the samples must be measured.

U-contents were established by fission-track analysis, using NBS reference glass standard SRM 614. Th, K and Rb contents were determined by INAA and K was independently measured by flame photometry.

An α -spectrometric determination of the $^{230}\text{Th}/^{234}\text{U}$ -disequilibria was not possible for the most useful samples (a)–(d) (directly related to the skull) because of the small amounts and U-content (see Table 2). U-series dating of the massive base travertine (e) yielded an age of $\geq 300,000$ yr. The growth of ^{230}Th (which is initially absent in speleothem) and its short-lived daughter nuclides (which are high-energy emitters) towards equilibrium results in a continuously increasing annual dose. Hence, the average annual dose was calculated iteratively with an equation derived by Wintle⁸. ^{222}Rn losses are taken to be negligible for all samples.

For ESR-dating of calcites, there have been no data on the efficiency of an α -radiation dose (relative to a β^- - or γ -dose) for the generation of unpaired, ESR-active electrons. We thus carried out this calibration on the calcite encrustation of the skull (a) by means of a standardized ^{241}Am α -source. The α -efficiency determined for sample (a) was $30 \pm 6\%$ and this value was also used for sample (b) in the same growth horizon.

The external γ -doses were measured by $\text{CaSO}_4 \cdot \text{Dy}$ TL-dosimeters (Harshaw TLD control 4378-32) (ref. 5). Dose rates

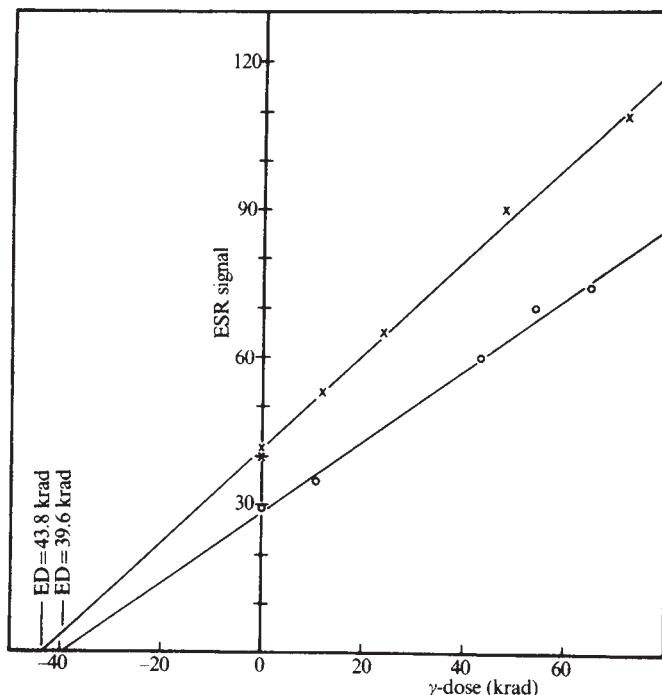


Fig. 2 Determination of the archaeological doses equivalent to ^{60}Co γ -irradiation (EDs) of the calcite encrustation (a) of the Petralona hominid skull from two sets of samples. $\times$, 150 K, 70 mg, calibration by Mn^{2+} -signals; $\circ$, 300 K, 150 mg, calibration by weight.

in Petralona Cave varied between 30 mrad yr^{-1} in some 20–30-cm deep boreholes in pure calcite floors and up to $\sim 200 \text{ mrad yr}^{-1}$ in clay-rich areas. At the site of discovery of the skull an external dose rate of $190 \pm 20 \text{ mrad yr}^{-1}$ was recorded on the surface of the travertine floor. The 1 mm polyethylene walls of the dosimeters containing the $\text{CaSO}_4 \cdot \text{Dy}$ partly absorb low-energy γ -rays and thus do not allow total γ -dose determinations. In addition, α - and β^- -radiation from airborne radionuclides (for example, radon and daughter products), which are not recorded by the $\text{CaSO}_4 \cdot \text{Dy}$ monitors, might have also affected the top layers. Hence, for the top layers (a) and (b) the given total dose-rate values may even be underestimated. The external dose rate 3–4 mm beneath the surface (as for samples (c) and (d)) was estimated to be 170 mrad yr^{-1} because of shielding which was confirmed by laboratory experiments. At various locations, 5–10 cm deep in the travertine floor dose rates varied between 70 and 110 mrad yr^{-1} . Thus a value of $90 \pm 20 \text{ mrad yr}^{-1}$ was taken for sample (e).

Although it cannot be proved for any ESR- or TL-dating, the external dose rate is commonly assumed to have remained

Table 2 Dose rates per year for the investigated samples (a)–(e)

	(a)	(b)	(c)	(d)	(e)
U-content (p.p.m.)	0.066	0.119	0.418	0.261	0.231
Mean* annual dose (mrad yr^{-1})	5.0 ± 0.4	9.0 ± 1.4	39.3 ± 16.0	24.5 ± 10.0	35.5 ± 14.5
Th-content† (p.p.m.)	1.84	1.39	0.3	≤ 0.1	≤ 0.1
Annual dose (mrad yr^{-1})	14.70	11.1	2.4	≤ 0.8	≤ 0.8
K-content (p.p.m.)	87	377	139	15	≤ 10
Annual dose (mrad yr^{-1})	0.9	4.0	1.2	0.1	≤ 0.1
Σ = internal dose rate (mrad yr^{-1})	$\Sigma 21 \pm 1$	$\Sigma 24 \pm 1$	$\Sigma 43 \pm 16$	$\Sigma 25 \pm 10$	$\Sigma 36 \pm 15$
External dose rate (mrad yr^{-1})	190 ± 20	190 ± 20	170 ± 20	170 ± 20	90 ± 20
Total dose rate (mrad yr^{-1})	211 ± 21	214 ± 21	213 ± 36	195 ± 30	126 ± 35

Internal dose rates are calculated from the U-, Th- and K-contents on the basis of the tables of Bell^{21,22}. Dose rate contribution by Rb $\leq 0.2 \text{ mrad yr}^{-1}$ throughout.

*The mean annual dose contributions by U-series nuclides take into account $^{234}\text{U}/^{238}\text{U}$ - and $^{230}\text{Th}/^{234}\text{U}$ -disequilibria of ~ 1.1 and ~ 0.85 , respectively, for samples (a)–(d), according to an age of $\sim 20,000$ yr. Secular equilibrium was determined for sample (e) by α -spectrometry. The α -efficiency of $30 \pm 6\%$ evaluated for sample (a) was also used for (b) of the same growth horizon. For samples (c), (d), and (e) the total range of α -efficiencies of 20% to 60% was applied, which have been evaluated in TL-dating of various stalagmites by Wintle⁸ and Bangert (personal communication).

† α -contributions to dose rates are neglected for Th-series, because the bulk of Th is in detrital aggregates (for example, in clay particles).

Table 3 Archaeological γ -equivalent doses (EDs), annual doses and ESR age date evaluated for the bone fragments from the Petralona hominid cranium (c) and interrelated calcite strata (a), (b), (d) and (e)

Sample	ED* (ESR) ($\times 10^3$ rad)	Annual dose (mrad yr $^{-1}$)	ESR age (kyr)
(a)	41.7 $\pm$ 4.2	211 $\pm$ 21	198 $\pm$ 40
(b)	41.8 $\pm$ 4.2	214 $\pm$ 21	195 $\pm$ 40
(c)	27.1 $\pm$ 2.7	213 $\pm$ 36	127 $\pm$ 35
(d)	38.6 $\pm$ 3.9	195 $\pm$ 30	198 $\pm$ 50
(e)	81.7 $\pm$ 12.3	126 $\pm$ 35	$\sim$ 650 $\pm$ 280

*Temperature of ESR-measurements is 150 K; linear regression coefficients of ESR growth curves ≥ 0.99 throughout.

†Mean value of 2 EDs (calibration by weight and Mn $^{2+}$ signals, respectively).

constant since the time of speleothem deposition. For the Petralona cranium this is probably true, because the place of discovery is at the head of a 'dead-end' branch of the cave², where there will be hardly any air movement.

The γ -dose rates monitored in Petralona cave by a portable NaI-scintillometer yielded smaller variations in the annual γ -doses (57–91 mrad yr $^{-1}$) (ref. 20) than those recorded by CaSO $_4$: Dy-monitors ($\sim$ 30–200 mrad yr $^{-1}$) (U. Bangert, personal communication). This is due to a higher spatial resolution of the CaSO $_4$: Dy-dosimeters in recording the γ -dose rates which generally show considerable variations (on a centimetre scale) vertical to the speleothem growth horizons.

From the total dose rates given in Table 2, we deduce absolute ages from the γ -equivalent archaeological doses (EDs) (see Table 3).

The ESR-ages of samples (a)–(d) are concordant within their limits of errors and sample (e), which is obviously much older, is not related to the hominid skull. The lower age of the bone fragments (c) compared with (a), (b) and (d) may be due to a partial recrystallization of the apatite crystal lattice of the bone.

Our ESR ages in Table 3 consistently indicate an age of $\sim$ 200,000 yr for the Petralona cranium. Taking into account the uncertainties of location, the technical difficulties and the controversial interpretations of the cranium, this result may clarify the phylogenetical position of the Petralona hominid, which lies between 160,000 and 240,000 yr BP.

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- Poulouanos, A. N. *Archaeology* **24**, 6–11 (1971).
- Poulouanos, A. N. *Anthropos* **7**, 7–11; 13–29 (1980).
- Sickenberg, O. *Geol. Geophys. Res. Inst. Athens* **9**, 1–16 (1964).
- Sickenberg, O. *Ann. Geol. Pays. Hellen.* **23**, 230–264 (1971).
- Hennig, G. J. thesis, Univ. Cologne (1979).
- Ikeya, M. *Anthropos* **7**, 143–151 (1980).
- Szwarcz, H. P., Liritzis, Y. & Dixon, A. *Anthropos* **7**, 152–173 (1980).
- Hennig, G. J., Bangert, U., Herr, W. & Poulouanos, A. N. *Anthropos* **7**, 215–241 (1980).
- Liritzis, Y. *Anthropos* **7**, 215–241 (1980).
- Ikeya, M. *Science* **207**, 977–979 (1980).
- Bangert, U. & Hennig, G. J. *PACT J.* **3**, 281–289 (1979).
- Wintle, A. G. *Can. J. Earth Sci.* **15**, 1977–1986 (1978).
- Zeller, E. J., Levy, P. W. & Mattern, P. L. *Proc. Symp. Radioactive Dating and Low Level Counting*, 531–540 (IAEA, Vienna, 1967).
- Ikeya, M. *Nature* **255**, 48–50 (1975).
- Ikeya, M. *Archaeometry* **20**, 147–158 (1978).
- Harmon, R. S., Thompson, P., Szwarcz, H. & Ford, D. *Nat. Speleol. Soc. Bull.* **27**, 21–33 (1975).
- Hennig, G. J., Bangert, U. & Herr, W. *Br. Mus. Occas. Pap.* **21**, 73–78 (1980).
- Szwarcz, H. P. *Archaeometry* **22**, 3–24 (1980).
- Wintle, A. G. *J. Electrostat.* **3**, 281–288 (1977).
- Liritzis, Y. & Poulouanos, A. N. *Anthropos* **7**, 252–259 (1980).
- Bell, W. T. *Archaeometry* **18**, 107–111 (1976).
- Bell, W. T. *Archaeometry* **21**, 243–245 (1979).

Isotopic evidence for prehistoric subsistence change at Parmana, Venezuela

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Recent archaeological results from Parmana, Venezuela, suggest that maize became a staple along the Orinoco River between about 800 BC and AD 400, contrary to prevailing views that maize was not important in prehistoric Amazonia. In maize, carbon dioxide is initially fixed as a C $_4$ carboxylic acid, rather than as phosphoglyceric acid as in C $_3$ plants. The organic carbon constituents of C $_4$ plants have a different $^{13}\text{C}/^{12}\text{C}$ ratio from C $_3$ plants, and this difference is transmitted to animals dependent on maize as a major carbon source. Thus, the $^{13}\text{C}/^{12}\text{C}$ ratio of animal remains is a reflection of their diet. Stable carbon isotope measurements on human skeletons from Parmana reported here show that a dramatic shift in the prehistoric diet from dependence on C $_3$ plants to C $_4$ plants (which include maize) did take place.

Students of Greater Amazonian cultural development commonly assume that the 'tropical forest' system was the major subsistence system of prehistoric times. In this system most calories are provided by cultivated manioc (that is, cassava) and most protein by hunting and fishing. In the Amazonian forests, where soils are poor and game scarce, tropical forest culture is associated with small, autonomous villages. The development of large, populous chiefdoms in the Amazon and Orinoco main-streams in late prehistoric times is usually attributed to the higher productivity of the floodplains for tropical forest subsistence^{1–3}.

An alternative argument holds that manioc cultivation and animal capture could not have supported dense populations in floodplains and that larger populations were based on the cultivation of seed crops, particularly maize⁴. Although maize is a minor crop in present Amazonian tropical forest societies, several ethnohistoric accounts^{5–8} describe it as an important staple in floodplain subsistence. Recent archaeological fieldwork in the Parmana area of the Orinoco basin, Venezuela, suggests that maize may have replaced manioc as the major staple along the Orinoco River during the Corozal phase, between about 800 BC and AD 400, and that the population increased 15-fold during this period.

To assess the archaeological and isotopic evidence for a change in prehistoric diet it is necessary to know the potential human food sources in the Parmana ecosystem, their carbon isotope ratios, and the potential productive capacity of the local tropical forest system as compared with one based on seed crop cultivation. The plants relevant to the discussion are all C $_3$ or C $_4$ plants, which have markedly different ^{13}C contents^{9–11}. Cumulative assessments of $^{13}\text{C}/^{12}\text{C}$ ratios in plant foliage, expressed as per mil (‰) deviations in ^{13}C ($\delta^{13}\text{C}$) from the Chicago PDB standard¹², yield mean values of -26.5% for C $_3$ and -12.5% for C $_4$ plants, with no overlap between their ranges of values^{13–15}. When animals and humans eat plants, these characteristic isotope ratios are registered in their tissues¹⁶, with further fractionation occurring during tissue formation^{17,18}. A fractionation value of $+5.1\%$ in human bone collagen, established for prehistoric hunter-gatherers in the North American woodlands¹⁹, is used here for calculations.

Maize (*Zea mays*) is a C $_4$ plant. A suitable average $\delta^{13}\text{C}$ value for maize kernels based on multiple measurements is not avail-

Table 1 $\delta^{13}\text{C}$ values of some manioc (*Manihot esculenta*) root products

Laboratory no.	Sample description	$\delta^{13}\text{C}$ (‰)
UCT-480	Manioc cake purchased in New York City; probable origin the Dominican Republic	-25.6
UCT-481	Same as UCT-480, another example	-26.0
UCT-483	Manioc root, Nkhata Bay, Malawi	-25.0

Precision of $\delta^{13}\text{C}$ is $\pm 0.1\%$.

able, so the average value for C_4 foliage (-12.5%) will be used here. Manioc (*Manihot esculenta*) is a C_3 plant, as shown by the $\delta^{13}\text{C}$ values in Table 1. The average for three samples from the Dominican Republic and Malawi is -25.5% , near the C_3 plant average. The samples were presumably cultivated in open fields, however, where the air is well mixed and the CO_2 has the $\delta^{13}\text{C}$ value of atmospheric CO_2 at -7% . In dense forests, restricted air circulation and CO_2 from rotting leaf litter combine to deplete the ^{13}C content of CO_2 available for photosynthesis²⁰. In the Amazonian rain forest, this canopy effect results in leaves of the upper canopy having $\delta^{13}\text{C}$ values of about -30% , while the undergrowth averages about -35% (ref. 21). The severity of the canopy effect can be expected to vary with forest density. Thus, the effect would be slightly less marked in tropical gallery forests, and less marked still in small forest clearings like swidden fields. No measurements are available for these situations, but it can be assumed that forest plants will have $\delta^{13}\text{C}$ values more negative than the C_3 plant average of -26.5% .

There are two major components to the Parmana ecosystem—the terrestrial habitats of gallery forest and savannah, and the seasonally fluctuating aquatic habitats of river, stream and lagoon (for detailed refs see ref. 4). Significant human food sources in the natural environment include fauna, a few tree and shrub products, and root plants. Plant material in the ecosystem is predominantly C_3 and forest derived, with the exception of floating meadows, which are C_3 but grow in the open, and savannahs, which have some C_4 grasses. The terrestrial fauna include a few grazers which may eat C_4 grasses, but are small in biomass and of relatively slow turnover. The aquatic fauna, especially the fish, make up the only substantial intensifiable human food source; they feed on forest-derived plant detritus. Thus, most carbon available to humans from wild sources is derived from C_3 plants and is strongly ^{13}C depleted; only a minuscule proportion of the carbon could come from C_4 plants.

The archaeological sequence consists of three ceramic traditions: La Gruta, Corozal and Camoruco^{4,22-26}. Excavations by Roosevelt in 1974 and 1975 provided information on pre-historic subsistence and population⁴. For La Gruta (2100–800 BC), tropical forest subsistence is indicated by ceramic griddles, small stone chips possibly from manioc graters, projectile points and poorly preserved faunal remains. Excavation produced carbonized plants apparently of wild species only (S.G. Stephens and H. Pinkley, personal communication). Population of the 500 km² area was small throughout the period, estimated at 120 or 0.24 per km² (based on a refuse area of 1.5 ha and comparison with ethnographic settlements). The subsistence of Corozal (~800 BC–AD 400) is characterized by the introduction of maize and transition to intensive maize cultivation, with continuation of tropical forest subsistence. Carbonized maize appears for the first time early in the period, becoming common by its end. Possible domestic legumes, as yet unidentified, occur in small quantities throughout Corozal, and possible maize-grinding tools appear. Estimated population for early Corozal is 560 or 1.1 per km² and 1,750 or 3.5 per km² for late Corozal (based on refuse areas of 7.5 and 23.3 ha, respectively). The Camoruco period (AD 400–1500) is characterized by intensive maize cultivation, with manioc cultivation and hunting and fishing probably making a small contribution to calories and protein. Maize remains were abundant, and Canavalia, a tropical bean, was identified (L. Kaplan, personal

communication). During this period population stabilized at about 1,800 or 3.6 per km², increasing to about 2,500 or 5 per km² between AD 1100 and 1500 (based on refuse areas of 24.1 and 34 ha, respectively).

The remains suggest a shift during Corozal from tropical forest subsistence, based on manioc, fish and game, to a system based primarily on maize, associated with a sizeable population increase. Maize is conclusively identified, based on carbonized plant remains (W. Galinat, personal communication), but identification of manioc is not conclusive, being based on presumed food-processing tools that could have been used for other foods²⁷. Manioc's presence will be unproven until direct botanical evidence can be found. Moreover, the archaeological finds do not establish the proportion of maize in the diet, due to poor preservation of other foods, especially manioc.

A shift from forest-based subsistence to a diet based on maize should produce a substantial change in the $^{13}\text{C}/^{12}\text{C}$ ratios of human tissues and should be detectable in bone collagen. Given a food web based primarily on plant material from the forest ($\delta^{13}\text{C}$ more negative than -26.5%) and enrichment in ^{13}C of 5.1‰ during collagen formation, human bone collagen should have a $\delta^{13}\text{C}$ value more negative than -21.4% . A hypothetical diet based entirely on maize or other C_4 plants (such as sorghum and millet) should yield bone collagen with a $\delta^{13}\text{C}$ value of -7.4% ; mixed diets should produce intermediate values.

Stable carbon isotope measurements were made on the five human skeletons recovered from the Corozal site (Table 2); two specimens dated to the inception of Corozal (~800 BC) and three to the Corozal/Camoruco interface (~AD 400). Collagen was extracted from the postcranial remains and converted to CO_2 in the Archaeometry Laboratory, University of Cape Town. The procedure involved slow decalcification in 2% HCl, washing in distilled water, vacuum drying and combustion. The bones were badly decomposed by the acid tropical soil, their appearance chalk-white and friable. All the postcranial remains of the child burial (UCT-267) had to be used to obtain the required 0.1 g of dried collagen. Mass spectrometric measurements of the $^{13}\text{C}/^{12}\text{C}$ ratios were made at the Pretoria Radiocarbon Laboratory and related to the Chicago PDB standard through a secondary standard. The results show a substantial isotopic difference between the early and late Corozal skeletons, the two early skeletons having an average $\delta^{13}\text{C}$ value of -26.0% , and the late group averaging -10.3% ; the individual values in each group are closely similar, presumably the result of stable subsistence systems.

The average $\delta^{13}\text{C}$ values of early and late Corozal diets can be calculated by subtracting the collagen enrichment value of 5.1‰ from the results in Table 2. The early Corozal diet averaged -31.1% , considerably more negative than the C_3 plant average.

Table 2 $\delta^{13}\text{C}$ values for collagen from human skeletons excavated at Corozal in the Parmana area

Laboratory no.	Provenience	$\delta^{13}\text{C}$ (‰)
Late Corozal, ~AD 400		
UCT-259	Skeleton 2 (adult), Exc. 3, level 18–22, prov. 543	-9.7
UCT-258	Skeleton 3 (adult), Exc. 3, level 18–22, prov. 544	-10.5
UCT-286	Skeleton 4 (adult), Exc. 3, level 18–22, prov. 544	-10.8
Average ($n = 3$)		-10.3
Early Corozal, ~800 BC		
UCT-260	Adult skeleton, centre burial, Exc. 2, level 16–17, prov. 293	-26.1
UCT-287	Child's skeleton, Exc. 2, level 18, prov. 545	-25.8
Average ($n = 2$)		-26.0

Early Corozal skeletons are associated archaeologically with a tropical forest subsistence system (probably manioc, fish and game), Late Corozal skeletons with maize, legumes and probably less manioc and animal food. Precision of $\delta^{13}\text{C}$ is $\sim 0.1\%$.

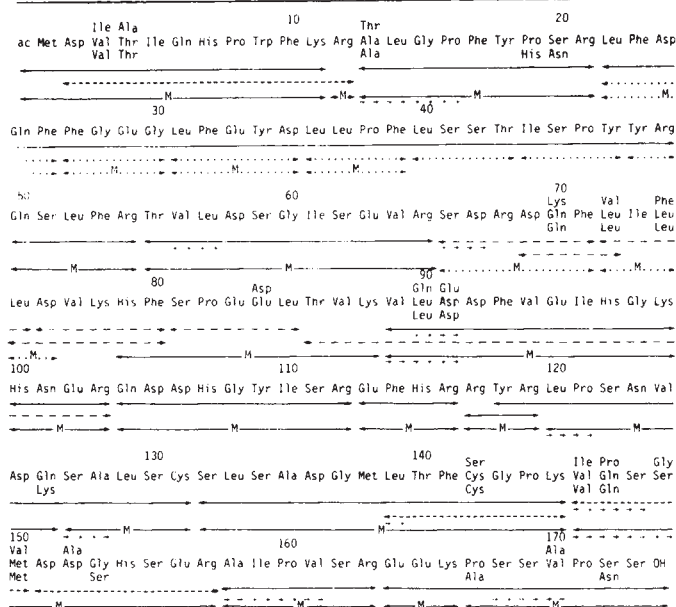


Fig. 1 Summary of sequence analysis of aardvark and manatee α -crystallin A chains. The central line represents the proposed sequence of aardvark α A. Residues which are different in the bovine α A chain¹⁸ are shown above the aardvark sequence and those residues in which the manatee α A chain differs from the bovine chain are given below the aardvark sequence. Positions 40-49 and 77-78 of the manatee sequence could not be determined, and a substitution Ser $\rightarrow$ Asn has occurred either at position 172 or 173. From three aardvark lenses and six manatee lenses, 36 mg and 12.5 mg, respectively, of α A chains could be isolated. The isolation and sequence analysis of the α A chains followed previously detailed methods¹⁹. The procedure is largely based on amino acid analyses of small peptides, which are compared with corresponding ones of the completely sequenced bovine α A chain¹⁸. When amino acid compositions of homologous peptides are found to be the same, it is assumed that their sequences are also. Peptides which differ in composition from corresponding bovine ones are, if possible, subjected to dansyl-Edman degradation to localize the positions of substitutions. The inaccuracy in the sequences so deduced is very small²⁰. This figure shows by different underlinings the peptides from which satisfactory amino acid analyses could be obtained. Peptides and residues within these peptides are mainly ordered by homology with the known bovine sequence¹⁸. The positions of a number of residues were determined by dansyl-Edman degradation ($\rightarrow$). Aardvark α A chain: Tryptic peptides ($\leftarrow\rightarrow$) were isolated by peptide mapping and gel filtration¹⁹. The large tryptic peptide 22-49 was further digested with thermolysin and the resulting small peptides ($\leftarrow\rightarrow$) isolated and analysed. From a tryptic digest of cyanogen bromide fragment (residues 2-138) was isolated peptide 2-12 ($\leftarrow\rightarrow$) and a large peptide containing residues 66-104. The latter peptide was decarboxylated and digested with chymotrypsin ($\leftarrow\rightarrow$), which resolved the sequence 66-88. Cyanogen bromide fragments corresponding to residues 139-150 and 151-173 were digested with trypsin. The resulting peptides ($\leftarrow\rightarrow$) covered the region 146-157. Manatee α A chain: Soluble tryptic peptides ($\leftarrow M \rightarrow$) were isolated by peptide mapping. A thermolytic digest of the water-insoluble tryptic core yielded peptides ($\leftarrow\rightarrow M \leftarrow\rightarrow$) which resolved residues 22-39 and the important region 66-76.

(pangolins)¹¹ and now the Tubulidentata. Although the relationship of Sirenia to the other paenungulates has not been questioned¹⁻⁷, we, nevertheless, report here the α A sequence of the Brazilian manatee (*Trichechus inunguis*) because it increases the body of relevant data on which to base our phylogenetic inferences.

α -Crystallin was isolated from three aardvark lenses and six manatee lenses, and the sequences of the α A chains were analysed as shown in Fig. 1. Comparison of the proposed aardvark and manatee α A sequences with the data set of other α A chains (Table 1) clearly reveals that both sequences share three or four apparently derived substitutions with hyrax and elephant: 70 Lys $\rightarrow$ Gln, 72 Val $\rightarrow$ Leu (not present in elephant), 74 Phe $\rightarrow$ Leu (also in the edentate *Tamandua*) and 142 Ser $\rightarrow$ Cys (also in man). No substitutions are uniquely shared with any other particular eutherian order. The aardvark and manatee α A chains each have in addition a relatively large number of unique substitutions: four in the aardvark (84 Glu, 149 Ser, 152 Asp, 170 Val) and five in the manatee (19 His, 20 Asn, 126 Lys, 167 Ala, 172 or 173 Asn).

To assess the sequence relationships of the aardvark and manatee α A chains more objectively and quantitatively, trees were constructed for the available α A sequences, using a maximum parsimony approach^{12,13}. The fewest possible number of nucleotide replacements (NRs) to arrange the 23 mammalian sequences, and the chicken and frog (*Rana esculenta*) sequences¹⁰ as outgroups, in an ancestral branching order, was found to be 133. Several equally parsimonious trees could be constructed which, however, all agreed in the positioning of the aardvark and the place of the paenungulates in relation to the other mammalian orders (Fig. 2). These most parsimonious trees clearly suggest a monophyletic origin of aardvark and paenungulates. Indeed, to separate the aardvark from the paenungulate branch, and place it at the base of the edentate line or as a separate branch next to edentates and paenungulates, costs an additional 4 NRs. All other positions are even less parsimonious. The most parsimonious solutions depict the elephant as the first offshoot of the paenungulates, due to the substitution 72 Val $\rightarrow$ Leu in hyrax, manatee and aardvark (Fig. 2). There is good morphological evidence to place elephant and manatee closest together¹⁻⁶. This would require one additional substitution, most probably a back substitution 72 Leu $\rightarrow$ Val in the elephant line. Faster evolving proteins, like myoglobin and haemoglobin, might provide better evidence on the phylogenetic relationships among the living paenungulate orders.

The suggestion that the Tubulidentata should be included in a monophyletic superorder Paenungulata is supported by some independent osteological and immunological findings¹⁴. Significant morphological resemblances of aardvark to hyraxes and elephants has already been noted¹⁵. Interestingly too, Tubulidentata, Hyracoidea, Sirenia and Proboscidea all seem to be indigenous African orders, which came into existence during the early Tertiary isolation of the continent⁵⁻⁷.

In agreement with our previous results^{10,11}, the Edentata and Paenungulata are consistently placed as the oldest offshoots from the main eutherian line (Fig. 2). This is mainly due to the absence in these orders of the substitutions 91 Asp $\rightarrow$ Glu and 153 Ser $\rightarrow$ Gly, shared by almost all other eutherian orders (Table 1). Such an early divergence of the edentates is in agreement with recent opinions about their phylogenetic position^{3,16}. More surprising is the ancient separation of a paenungulate branch, far from the ungulates. It would in fact cost at least 5 additional NRs to place the paenungulates and aardvark in the ungulate-cetacean region of the tree. Although the paenungulates are usually grouped among the ungulate orders, Romer⁵ states that it is "far from certain that they form a single natural group".

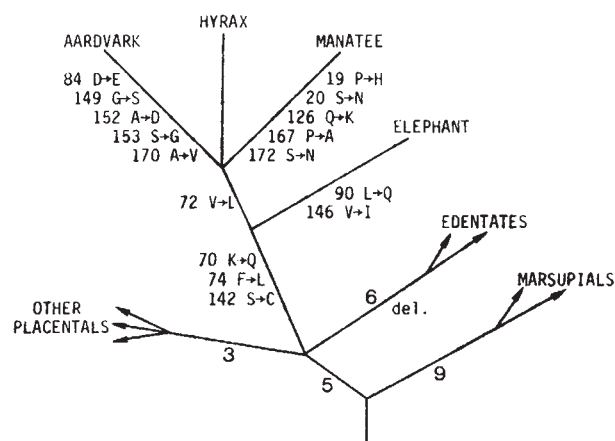


Fig. 2 The aardvark-paenungulate region of the most parsimonious phylogenetic trees constructed on the basis of α -crystallin A sequences from 23 mammalian species (Table 1), and from chicken and frog as outgroups. Several equally parsimonious trees are possible, which differ in the precise branching pattern of the other placental orders. The inferred amino acid substitutions in the aardvark and paenungulate lines are indicated. Numbers of nucleotide replacements are given in neighbouring branches. A deletion of three residues occurs in the edentate α A chains.

No other protein sequence data are available from aardvark, hyrax and manatee to corroborate our proposals. However, the sequences of elephant β -haemoglobin, myoglobin and fibrinopeptides are known and also fail to group the Proboscidea with the ungulates¹⁷.

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1. Simpson, G. G. *Bull. Am. Mus. nat. Hist.* **85**, 1–350 (1945).
2. Van Valen, L. *Evolution* **25**, 420–428 (1971).
3. McKenna, M. C. in *Phylogeny of the Primates* (eds Luckett, W. P. & Szalay, F. S.) 21–46 (Plenum, New York, 1975).
4. Szalay, F. S. in *Major Patterns in Vertebrate Evolution* (eds Hecht, M. K., Goody, P. C. & Hecht, B. M.) 315–374 (NATO Advanced Study Institute, Plenum, New York, 1977).
5. Romer, A. S. *Vertebrate Paleontology* 3rd edn (University of Chicago Press, 1966).
6. Thénius, E. *Phylogenie der Mammalia* (de Gruyter, Berlin, 1969).
7. Patterson, B. in *Evolution of African Mammals* (eds Maglio, V. J. & Cooke, H. B. S.) 268–278 (Harvard University Press, 1978).
8. Bloemendal, H. (ed.) *Molecular and Cellular Biology of the Eye Lens* (Wiley-Interscience, New York, 1981).
9. Clayton, R. M. in *The Eye* Vol. 5 (ed. Davson, H.) 399–494 (Academic, New York, 1974).
10. De Jong, W. W., Gleaves, J. T. & Boulter, D. J. *molec. Evol.* **10**, 123–135 (1977).
11. De Jong, W. W., Zweers, A., Joysey, K. A., Gleaves, J. T. & Boulter, D. in *The Evolution and Ecology of Sloths, Anteaters and Armadillos* (ed. Montgomery, G. G.) (Smithsonian Institution Press, Washington DC, in the press).
12. Moore, G. W., Barnabas, J. & Goodman, M. J. *theor. Biol.* **38**, 459–485 (1973).
13. Moore, G. W. in *Molecular Anthropology* (eds Goodman, M. & Tashian, R. E.) 117–137 (Plenum, New York, 1976).
14. Shoshani, J., Goodman, M. & Prychodko, W. *Am. Zool.* **18**, 601 (1978).
15. Le Gros Clark, W. E. & Sonntag, D. F. *Proc. zool. Soc. Lond.* **30**, 445–485 (1926).
16. Engelmann, G. F. in *The Evolution and Ecology of Sloths, Anteaters and Armadillos* (ed. Montgomery, G. G.) (Smithsonian Institution Press, Washington DC, in the press).
17. Dene, H., Goodman, M. & Romero-Herrera, A. E. *Proc. R. Soc. B* **207**, 111–127 (1980).
18. Van der Ouderaa, F. J., De Jong, W. W. & Bloemendal, H. *Eur. J. Biochem.* **39**, 207–222 (1973).
19. De Jong, W. W. & Terwindt, E. C. *Eur. J. Biochem.* **67**, 503–510 (1976).
20. Van Druten, J. A. M., Peer, P. G. M., Bos, A. B. H. & De Jong, W. W. *J. theor. Biol.* **73**, 549–561 (1978).

Experience required for pheromone recognition by the apple maggot fly

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Remarkable progress has been made during the past 20 yr in the identification of chemical components and receptor sites of pheromones of various insects. However, our understanding of the physiological and ecological parameters which affect the response of individual insects to pheromones remains rather limited. Here we report that in apple maggot fly, *Rhagoletis pomonella*, females require experience with oviposition-determining pheromone (ODP) before they are able to discriminate between ODP-marked and unmarked host fruit. This seems to be the first conclusive evidence in an insect that pheromone recognition may depend on previous contact with the particular pheromone.

After egg laying in the fruit flesh, a *R. pomonella* female deposits ODP by dragging its ovipositor on the fruit surface¹. One bout of dragging on a hawthorn (*Crataegus* sp.) fruit of ~5 mm diameter deters the same or other females from ovipositing in that fruit in the field, although the degree of deterrence is reduced in the laboratory. Prokopy¹ suggested that the ODP elicits uniform egg distribution among larval food resources, a character which has high survival value in so far as only a limited number of larvae can mature in a single fruit.

We found that recently matured females deprived of host fruit until testing did not discriminate between ODP-marked and

unmarked hosts. In contrast, females that were allowed access to ODP-marked host fruit 1 day before testing generally rejected ODP-marked fruit (Table 1, expt 1). To determine whether these results stemmed from a difference between females in oviposition deprivation or to a difference in previous contact with ODP, we placed newly matured wild flies singly in cages and treated each fly in one of two ways.

In treatment A, each fly was presented with and allowed to oviposit in one clean (no ODP), uninfested hawthorn fruit. Immediately after oviposition, the fly was transferred using a small triangular piece of clean filter paper to a clean uninfested apple, where it was allowed to deposit ODP. We directed the fly's course of dragging on this much larger fruit so that it never contacted its own pheromone trail with its pheromone receptors (tarsal D-hairs)^{2,3}. We repeated this procedure twice more at 45-min intervals on day 1. On day 2, we again repeated the procedure twice. Forty-five minutes after the second oviposition, we presented the fly with a 5-mm diameter hawthorn fruit marked with ODP from two dragging bouts by other females.

Treatment B flies were also allowed to oviposit in three hawthorn fruits and drag on three apples on day 1. However, the second and third hawthorn fruits offered were marked with ODP from three previous (~1 h earlier) dragging bouts. If the flies rejected the marked fruit, they were presented with clean fruit for oviposition. The procedure followed on day 2 was the same as for treatment A flies.

Treatment B (experienced) flies generally rejected ODP-marked fruit and accepted clean ones. Treatment A (naive) flies readily accepted ODP-marked fruit (Table 1, expt 2a). In addition, significantly more treatment A than treatment B flies rejected clean fruit presented to them after their rejection of a marked fruit. This experiment was repeated, with similar though less dramatic results (Table 1, expt 2b).

Prokopy⁴ reported that barometric pressure may affect the level of activity and ODP discrimination ability of apple maggot flies. In expt 2a and b we found that more flies completed the experimental protocol on days with moderate or high barometric pressure (≥ 29.7 mbar). When we reexamined data for expt 2b including only data from days in which we were able to test to completion at least four naive and four experienced flies, 71% ($n = 14$) of the naive flies accepted ODP-marked fruit compared with 40% ($n = 10$) which accepted on days when we were unable to test four naive and four experienced flies.

To determine whether flies could obtain necessary pheromone experience by contacting their own ODP trail while dragging, we repeated the experimental protocol, but did not transfer the flies to apples for dragging. All flies contacted their own trail an average of 4.4 times (per fruit) while dragging. In this case, treatment A flies rejected ODP-marked fruit as readily as did treatment B flies (Table 1, expt 3).

In a final test, we withheld experienced flies from ODP for 96 h (flies were allowed to oviposit and ODP drag, but not contact any ODP, three times on each of the 4 days) and found that they rejected ODP-marked fruit as readily as did recently experienced flies, thus demonstrating the retention of ability to recognize ODP for at least 4 days (Table 1, expt 4). Klomp *et al.*⁵ reported that *Trichogramma embryophagum*, a hymenopteran parasite of lepidopteran larvae, forgets and must relearn to discriminate between parasitized and non-parasitized hosts. However, they suggested that their results could be explained by the increasing tendency of the wasps to oviposit at increasing time of deprivation from oviposition sites.

Alcock's definition⁶ of 'restricted learning', that is, 'an animal acquires a limited piece of information from the environment that changes the behaviour of the animal in a precise manner', can be applied to the ability to recognize ODP by apple maggot flies. Electrophysiological tests show that the ODP receptors on the tarsi fire the first time they contact ODP (L. Bowdan, V. Dethier and R.J.P., unpublished data). However, our results show that such a message is not translated into a rejection response in naive flies. This mechanism may provide flies with a means of reducing the cost of continually maintaining an unused

Table 1 Response of apple maggot flies to ODP-marked hawthorn fruit before and after experience with ODP

Experiment	Treatment	n	% Rejection of ODP-marked fruit*	n	% Rejection of clean fruit after rejection of an ODP-marked fruit
1	No previous host or ODP experience	17	17.6 ($P \leq 0.001$)	5	40.0 (NS)
	Previous fruit and ODP experience	20	60	14	14.3
2a	Treatment A (naive)	29	13.8 ($P \leq 0.001$)	17	76.5 ($P \leq 0.001$)
	Treatment B (experienced)	28	64.3	20	10.0
2b	A	24	41.6 ($P \leq 0.08$)	17	41.2 ($P \leq 0.07$)
	B	27	66.7	21	14.3
3	A	16	75.0 (NS)	23	47.8 ($P \leq 0.02$)
	B	18	88.8	18	11.1
4	96-h ODP deprivation	16	62.5 (NS)	10	20.0 (NS)
	No ODP deprivation	16	56.3	9	22.2

* If a fly rejected ODP-marked fruit, we presented it with a clean fruit. If the fly also rejected the clean fruit we disqualified it from this analysis. NS, not significant.

information processing system, because flies may not encounter ODP-marked fruit in conditions of high fruit density, low fly population or when immature. However, once mature flies have oviposited in a single small hawthorn fruit (= native host fruit) they may gain, through tarsal contact with their own ODP trail, the pheromonal experience needed to activate the system.

We are carrying out further experiments to determine why naive apple maggot flies reject clean fruit more often after encountering ODP than do experienced flies.

We suggest that restricted learning of pheromone recognition may be more widespread than is believed. For example, Cammaerts-Tricot⁷ and Le Moli and Passetti⁸ suggested, but did not prove, that perception of pheromones by *Myrmica* and *Formica* ants, respectively, depends on experience, and Vinson *et al.*⁹ demonstrated associative learning of kairomonal ovipositional cues by *Bracon mellitor*, a parasitic wasp. van Lenteren and Bakker¹⁰ first demonstrated that *Pseudeucoila bochei*, a

hymenopteran parasite of *Drosophila* larvae, must 'learn' to discriminate against parasitized hosts. Their results strongly suggest that the key component in the learning process is the marking pheromone deposited by *P. bochei* after oviposition. However, because the parasite marks its hosts internally, van Lenteren and Bakker were unable to demonstrate pheromonal contact. Our study parallels their pioneering work and provides the first unequivocal evidence for pheromone learning in insects.

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1. Prokopy, R. J. *Envir. Ent.* **1**, 326-332 (1972).
2. Prokopy, R. J. & Spatcher, P. *Ann. ent. Soc. Am.* **70**, 960-962 (1977).
3. Crnjar, R., Dethier, V. G. & Prokopy, R. J. *Proc. N.Y. ent. Soc.* **86**, 283-284 (1978).
4. Prokopy, R. J. in *Management of Insect Pests with Semiochemicals* (ed. Mitchell, E. R.) (Plenum, New York, in the press).
5. Klomp, H., Teerink, B. J. & Wei Chun, M. *Neth. J. Zool.* **30**, 254-277 (1980).
6. Alcock, J. *Animal Behaviour* (Sinauer, Sunderland, 1979).
7. Cammaerts-Tricot, M. C. *Insectes soc.* **21**, 235-248 (1974).
8. Le Moli, F. & Passetti, M. *Boll. Zool.* **45**, 389-398 (1978).
9. Vinson, S. B., Barfield, C. S. & Henson, R. D. *Phys. Ent.* **2**, 157-164 (1977).
10. van Lenteren, J. C. & Bakker, K. *Nature* **254**, 417-419 (1975).

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Behavioural and microspectrophotometric measurements of colour vision in monkeys

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Inherited abnormalities of colour vision are most commonly attributed to abnormalities of the photosensitive pigments in the cone cells of the retina^{1,2}. Here, we describe direct microspectrophotometric measurements on the eyes of two squirrel monkeys whose colour vision had been shown to differ behaviourally. Our results are consistent with classical explanations of abnormal colour vision.

Some colour-deficient human observers are dichromats, which means that they are able to match any colour with a mixture of two primary lights, whereas the normal, trichromatic observer requires three variables. It is exactly two hundred years since von Gentilly³⁻⁵ advanced the most natural explanation of this condition, that the dichromat lacks one of the three types of retinal receptor enjoyed by the normal trichromat. It is also one hundred years since Lord Rayleigh described⁶ a different

abnormality of colour vision, anomalous trichromacy. Like the normal, the anomalous observer requires three variables in colour matching, but in matching a mixture of red and green to a monochromatic yellow light (the 'Rayleigh match') he needs either more red (protanomalous) or less red (deutanomalous) than the normal. This has been explained by supposing that the anomalous retina contains three types of photopigment but that the absorbance curve for one of them is displaced along the spectrum from its normal position^{1,2,7-11}. Despite much work, the exact relationships between the various types of colour vision and retinal photopigments are far from settled. Two recent developments have led to the present report. First, it has been shown that among a population of squirrel monkeys (*Saimiri sciureus*) there occur clear variations in colour vision^{12,13}. Second, the technique of microspectrophotometry¹⁴⁻²⁰, in which a narrow, monochromatic, measuring beam is passed through isolated photoreceptors, has advanced to a stage where it is possible to characterize the types of receptor within an individual primate retina and thus relate the results to earlier behavioural measurements on the same subject.

The squirrel monkeys examined in the present study were both adult females of the subtype exported through Iquitos, Peru²¹; they were drawn from a larger group trained on colour vision tests in Santa Barbara, California. The behavioural tests^{12,13} were all conducted in a forced-choice discrimination apparatus in which the monkeys viewed three circular, trans-illuminated panels. They were taught to touch one of the three panels which was illuminated differently from the other two, in order to receive a 97-mg banana-flavoured food pellet. Which of

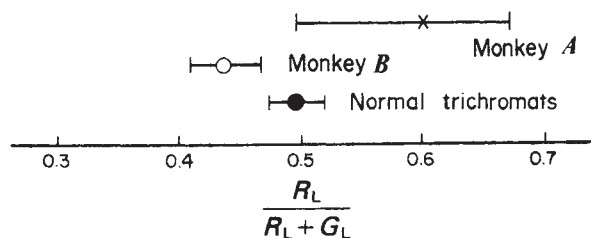


Fig. 1 Rayleigh match results from squirrel monkeys and humans. The match values are expressed as the ratio of the luminance of a red (624 nm) light (R_L) to the sum of the luminances of the red and green (536 nm) lights ($R_L + G_L$) needed in a mixture to match an equiluminant yellow (585 nm) light. The solid circles give the average values for 20 normal human trichromats, so categorized by their performances on the Farnsworth-Munsell 100-hue test. The horizontal line shows the total range of matches made by this sample of subjects. The results for the two monkeys were obtained from tests in which they were forced to discriminate between a red/green mixture and a yellow. Across repeated test sessions, 21 different mixture combinations were tested. The large symbols represent the midpoints of the range of mixtures over which they were unable to discriminate various red/green combinations from yellow; the horizontal bars enclose the mixture range over which their discrimination performances were not significantly different from chance (95% confidence level).

the three panels was positive (that is, differently illuminated) varied randomly from trial to trial.

Results from three tests of vision are reported. First, detection thresholds were measured for 540 and 640 nm monochromatic test lights superimposed on achromatic backgrounds (3 cd m^{-2}). One animal (A) was 1.27 log units less sensitive to the 640 nm light than to 540 nm, whereas the other (B) was much more sensitive to the long-wavelength light, showing a threshold difference of only 0.36 log units. Sensitivity variations of this magnitude are characteristic of this species¹².

Two further tests specifically examined colour vision. The first was a Rayleigh match assessing the relative proportions of mixed red (625 nm) and green (536 nm) light that the animal was unable to discriminate from a yellow (585 nm) (Fig. 1). Human subjects were tested in the same apparatus and the matches (mean and total range) obtained from 20 normal trichromats are shown in Fig. 1 for comparison. For the monkeys, the symbols represent the midpoints of the range over which they were unable to discriminate the red/green mixture from the yellow and the horizontal bars show the mixture range over which their discrimination was not significantly different from chance. It will be seen that monkey B required somewhat more green light in the mixture than any of the normal human trichromats whereas monkey A required much more red. Note also that the mixture range that could not reliably be discriminated from yellow was very much larger for subject A.

In another experiment we measured, at each of 10 spectral locations, how much the wavelength of a test light had to be shifted along the spectrum for the animal to discriminate a wavelength difference correctly. The abilities of the two monkeys to distinguish wavelengths were very similar for values from 450 to ~540 nm (Fig. 2). At longer wavelengths, monkey B continued to show relatively good discrimination, but A's discrimination worsened strikingly.

If we categorize the animals in terms of human vision, then monkey A was a protan; we leave open the question of whether she was an extreme protanomal or a true dichromat. Monkey B requires significantly more green light in the Rayleigh match than the normal human trichromat, and we describe her as deuteranomalous, but she is less aberrant in this regard than the typical human deuteranomalous observer^{1,2}.

The behavioural results were not known to the microspectrophotometrists (J.K.B. and J.D.M.), nor the microspectrophotometric data to G.H.J., until the two sets of results had been handed to an independent third party.

The monkeys were flown to Britain, where microspectrophotometric measurements were made with a modified Liebman microspectrophotometer^{16,22} under computer control. The preparation of tissue was as described for *Macaca fascicularis* by Bowmaker, Dartnall and Mollon¹⁹. Several samples were

taken from each retina. The microspectrophotometer was programmed to step from 700 to 390 nm in 2-nm steps, taking wavelengths with even values, and to return taking the interleaved wavelengths. A total of 34 individual records were obtained from animal A, and 47 from B.

Figure 3 shows for each animal (1) the mean absorbance curve for each class of photoreceptor, excluding short-wave cones, which were too few to provide mean spectra, and (2) the distribution of peak sensitivities for all individual records. From animal A we recorded 2 violet-sensitive receptors (mean $\lambda_{\text{max}} = 431.3 \text{ nm}$), 13 rods (mean $\lambda_{\text{max}} = 496.4 \text{ nm}$, s.d. = 4.69) and 19 green-sensitive receptors (mean $\lambda_{\text{max}} = 535.4 \text{ nm}$, s.d. = 3.45); the longest λ_{max} estimated for any cell of this animal was 542.5 nm. The mean and standard deviation for the P535 receptors closely resemble those for the middle-wavelength receptors of macaques¹⁸⁻¹⁹. Because animal A seems to have only one photopigment in the red-green range, its behavioural sensitivity at 640 nm relative to that at 540 nm should be directly predictable from the microspectrophotometric measurements. From the mean absorbance values at the two wavelengths we estimated log absorbance¹⁹ for an axial beam, assuming an outer segment length of $30 \mu\text{m}$ and a pigment density of $0.015 \mu\text{m}^{-1}$ and assuming that absorption by the ocular media is identical at 540 and 640 nm. This calculation predicts a difference in log sensitivity of 1.23, very close to the obtained value of 1.27.

From monkey B we recorded one short-wave receptor ($\lambda_{\text{max}} = 427 \text{ nm}$), 24 rods (mean $\lambda_{\text{max}} = 501.7 \text{ nm}$, s.d. = 3.58), no receptors in the vicinity of 535 nm and a broad range of receptors with peak sensitivities between 546 and 577 nm. There is no overlap between the long-wavelength receptors from this animal and the P535 receptors from the protan, a highly significant difference ($z = 5.63$, Mann-Whitney U -test).

The long-wave receptors of animal B, taken as a whole, have a higher standard deviation (8.17 nm) than we should typically expect for a single class of photoreceptor in an individual primate; and *prima facie* they fall into two groups, with a gap in the distribution at 562 nm (Fig. 3d). The two groups have means of 552.2 nm ($n = 16$) and 568.2 nm ($n = 9$). The latter value can be compared with the mean of 567.0 nm obtained for the long-wavelength receptors of *Macaca fascicularis*¹⁹. Our confidence in the existence of more than one long-wavelength pigment in this animal is reinforced by the fact that the mean absorbance spectra for the two subgroups (Fig. 3c) show very similar absorbance at short wavelengths and seem to have a constant separation; we should not expect this to be the case if, say, the large variation in λ_{max} arose from variations in optical

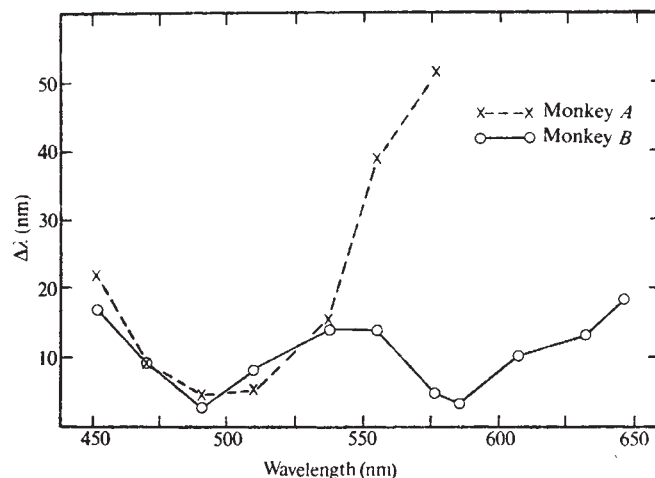


Fig. 2 Wavelength discrimination functions for two squirrel monkeys. The wavelength discrimination values ($\Delta\lambda$) indicate the magnitude of the wavelength change required at each spectral location to support discrimination between two equiluminant spectral lights at a criterial level of 70% correct. The values are averages for differences in both spectral directions except at 452 nm where the change could only be measured towards the longer wavelengths.

scattering or in the presence of photoproducts at short wavelengths, which would distort the absorbance spectrum.

As in macaques and man^{19,20}, the short-wave receptors seem to be rare in squirrel monkeys. In the case of the rods there is a 5-nm difference between animals in $\lambda_{\max}$, a difference which is associated with a small difference in absorbance at short wavelengths; we do not know whether the latter difference reflects short-wave contaminants or a true difference in the absorbance spectrum.

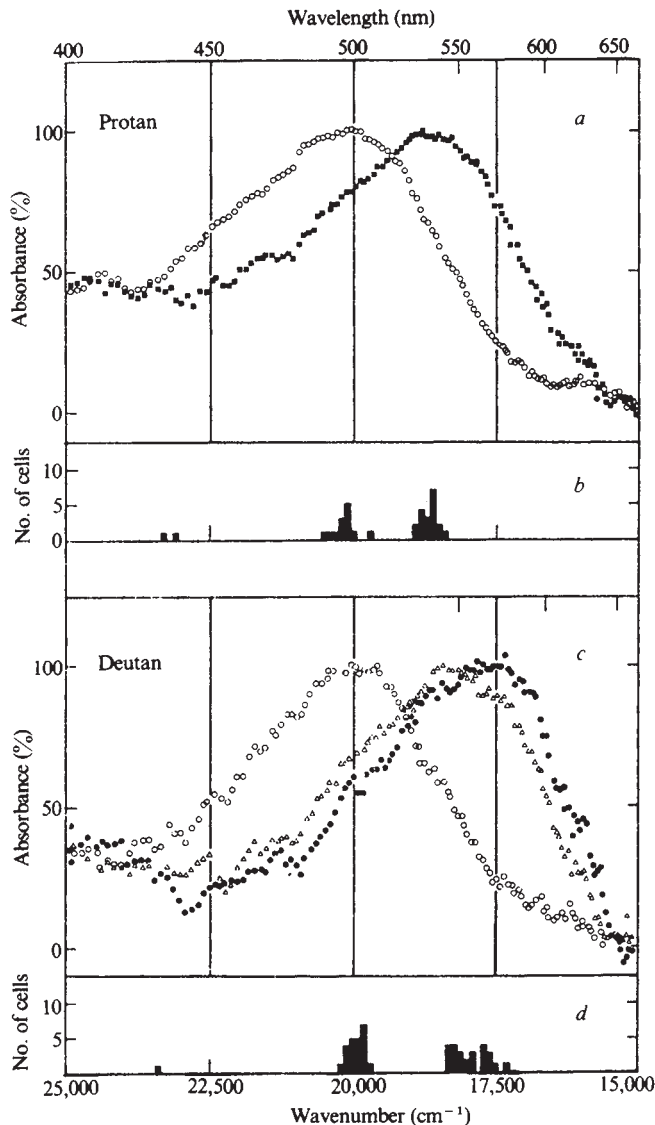


Fig. 3 *a, c*, Mean absorbance spectra for different classes of photoreceptor in two individual squirrel monkeys. Each datum point corresponds to the average of values obtained at two adjacent wavelengths, one value recorded in the descending scan (see text) and the other recorded in the ascending scan. The absorbance spectra for individual receptors were not normalized before averaging. The mean spectra have been normalized to have a maximum of 100%. *b, d*, Distribution of values of peak sensitivity of individual receptors from the protan (*b*) and deutan (*d*) animals. The bin size is 100 cm⁻¹. With the exception of the three short-wavelength receptors, values of peak sensitivity were derived as follows. The raw absorbance values for individual wavelengths were smoothed using an 11-nm running average. The approximate peak of this curve was estimated and each of 50 individual (smoothed) absorbance values on either side of the provisional peak was referred to an appropriate nomogram to estimate the wavenumber of peak sensitivity; this operation amounts to finding where the nomogram must be located on a wavenumber abscissa to yield the absorbance value under consideration. The mean of the many separate estimates for a given cell is the value entered in the histogram. This method resembles that described by Bowmaker *et al.*¹⁹, except that the smoothing and subsequent analysis are carried out by the computer and more individual estimates are used. For the three short-wavelength records we estimated wavenumber of peak sensitivity directly from the 2-nm averages (see above) in the restricted range 400–475 nm, using the frog green-rod nomogram²².

This first microspectrophotometric study of behaviourally different conspecifics is consistent with classical explanations of colour deficiency and anomaly. Within the limits of our sampling, the severely protan animal lacks entirely the long-wave receptors found in macaques^{18,19}. The deuteranomalous animal lacks the P535 receptors found in the protan but probably has more than one type of receptor in the range 546–577 nm.

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1. Boynton, R. M. *Human Color Vision* (Holt, Rinehart & Winston, New York, 1979).
2. Pokorny, J., Smith, V. C., Verriest, G. & Pinckers, A. J. L. G. *Congenital and Acquired Color Vision Defects* (Grune & Stratton, New York, 1979).
3. Voigt, J. H. *Gothaisches Magazin für das Neueste aus der Physik und Naturgeschichte* Vol. 1 (ed. Lichtenberg, L. C.) 57–61 (Ettinger, 1781).
4. Palmer, G. *Théorie de la Lumière, Applicable aux Arts et Principalement à la Peinture* (Hardouin & Gattey, Paris, 1786).
5. Walls, G. L. *J. hist. Med.* **11**, 66–96 (1956).
6. Rayleigh, Lord *Nature* **25**, 64–66 (1881).
7. von Kries, J. in *Handbook of Physiological Optics* Vol. 2 (von Helmholtz, H.) (translated by Southall, J. P. C.) (Optical Society of America, 1924).
8. Hurvich, L. M. & Jameson, D. *Documenta Ophthalm.* **16**, 409–442 (1962).
9. Alpern, M. & Torii, S. *J. gen. Physiol.* **52**, 717–737, 738–749 (1968).
10. Rushton, W. A. H., Powell, D. S. & White, K. D. *Vision Res.* **13**, 2017–2031 (1973).
11. MacLeod, D. I. A. & Hayhoe, M. J. *Opt. Soc. Am.* **64**, 92–96 (1974).
12. Jacobs, G. H. *Science* **197**, 499–500 (1977).
13. Jacobs, G. H. & Blakeslee, B. *Invest. Ophthalm. vis. Sci. Suppl.* **136** (1980).
14. Marks, W. B., Dobelle, W. H. & MacNichol, E. F. *Science* **143**, 1181–1183 (1964).
15. Brown, P. K. & Wald, G. *Science* **144**, 45–51 (1964).
16. Liebman, P. A. & Entine, G. J. *Opt. Soc. Am.* **54**, 1451–1459 (1964).
17. Dobelle, W. H., Marks, W. B. & MacNichol, E. F. *Science* **166**, 1508–1510 (1969).
18. Bowmaker, J. K., Dartnall, H. J. A., Lythgoe, J. N. & Mollon, J. D. *J. Physiol., Lond.* **274**, 329–348.
19. Bowmaker, J. K., Dartnall, H. J. A. & Mollon, J. D. *J. Physiol., Lond.* **298**, 131–143 (1980).
20. Bowmaker, J. K. & Dartnall, H. J. A. *J. Physiol., Lond.* **298**, 501–511 (1980).
21. Cooper, R. W. in *The Squirrel Monkey* (eds Rosenblum, L. A. & Cooper, R. W.) 1–29 (Academic, New York, 1968).
22. Knowles, A. & Dartnall, H. J. A. *The Photobiology of Vision* (Academic, New York, 1977).

Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque

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Study of parallel processing in the visual pathway¹ of the cat has revealed several classes of retinal ganglion cells which are physiologically distinct and which project to various locations in the brain^{2,3}. Two classes have been studied most extensively: X cells, which sum neural signals linearly over their receptive fields, and Y cells, in which the spatial summation is nonlinear^{1,4}. In the cat's lateral geniculate nucleus (LGN) cells also can be classified as X or Y, a result of the parallel projection of retinal X and Y inputs to different geniculate neurones^{5–9}. We report here our study of parallel signal processing in the LGN of the macaque monkey. We find that (1) monkey LGN cells can be classified as X or Y on the basis of spatial summation; (2) X-like cells are found in the four parvocellular and the two magnocellular laminae, whereas Y-like cells are found almost exclusively in the magnocellular laminae; and (3) the cells of the magnocellular laminae have high sensitivity and the parvocellular cells low sensitivity for homochromatic patterns. This implies that in macaque monkeys the magnocellular cells and their cortical projections may be the neural vehicle for contrast vision near threshold. The cells of the parvocellular laminae seem to be primarily concerned with wavelength discrimination and patterns of colour. As the human visual system is similar to that of the macaque in structure and behavioural performance, our findings are probably also applicable to man.

In macaque monkeys, as in man, the LGN is a highly organized, layered nucleus. There are four dorsal layers of small

cells (parvocellular) and two ventral layers of large cells (magnocellular). Previous investigators¹⁰⁻¹² concluded that there was a complete segregation of X and Y cells in the monkey's geniculate nucleus, the X cells residing in the parvocellular laminae and the Y cells in the magnocellular laminae. These conclusions depended on identifying X and Y cells by the time course of response to visual stimulation and the latency of response to electrical stimulation of the optic chiasm. Although such characteristics have been used to classify X and Y cells in the cat LGN^{6,7}, a correlation between response time course and linearity of spatial summation has not been established for neurones of the monkey's LGN.

In our experiments we used nine cynomolgus monkeys (*Macaca fascicularis*) anaesthetized with urethane (20 mg per kg per h) and paralysed with Flaxedil. The activity of single cells was recorded extracellularly using glass micropipettes (tip resistance, 10–30 M Ω). In about half the penetrations the pipettes were filled with a saturated solution of Fast Green dye in Ringer's solution to mark the position of the recording electrode. Electrode tracks were reconstructed from histological sections by using the dye marks and distances traversed along the track. This was important for assigning a given cell to a particular geniculate lamina because laminar boundaries are often irregular and the layer 3–layer 2 boundary, which is the critical parvocellular–magnocellular boundary, is not accompanied by an eye change. Visual stimuli were produced electronically on the face of a CRT¹³ which subtended 10° by 8° in

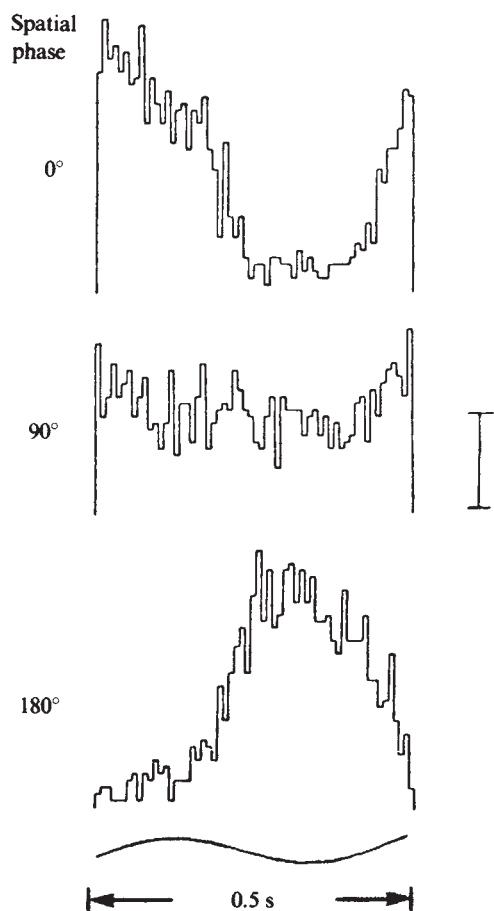


Fig. 1 Response of an X cell from a magnocellular layer at three different positions (spatial phases). The cell was recorded in layer 2 of the macaque's LGN. The stimulus was a sine grating of 1.5 cycle deg⁻¹, 50% contrast, contrast reversed with a sinusoidal time course at 2 Hz as indicated at the bottom of the figure. 0° and 180° were spatial phases at which the stimulus produced maximal responses; 90° was the spatial phase of the null response. The vertical calibration, drawn close to the middle histogram, is 30 impulses per second.

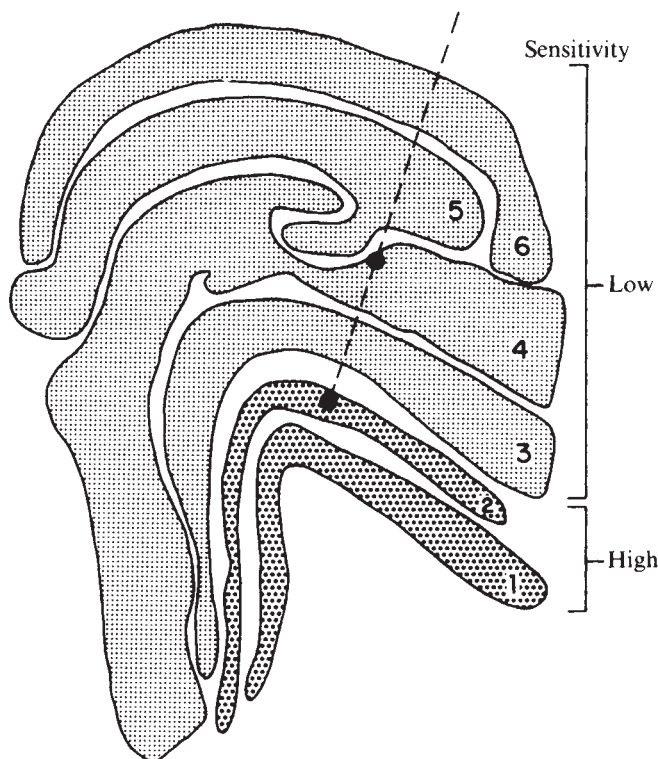


Fig. 2 Contrast sensitivity in the macaque's LGN. This drawing is traced from a single 100- μ m thick section of the LGN. The parvocellular laminae are numbered 6, 5, 4 and 3 and the magnocellular laminae 2 and 1. The parvocellular cells all had lower contrast sensitivity than did the X and Y cells of the magnocellular laminae. A reconstructed electrode track is shown; the two dark circles represent green dots left by the electrode at sites where (1) a typical parvocellular X cell of low sensitivity was recorded in layer 4 and (2) a typical magnocellular X cell of high contrast sensitivity (>50) was recorded in layer 2. The latter was the first cell of high contrast sensitivity found on this track.

visual angle. In some experiments a CRT with a P31 (green) phosphor was used, in others a CRT with a P4 (white) phosphor with no difference in results. The mean luminance was 20–60 cd m⁻², in the low photopic range. The pupils were dilated to ~6-mm diameter and the eyes protected with clear plastic contact lenses. In several experiments Wratten colour filters (no. 26, red; no. 61, green; no. 48A, blue) were placed in front of the CRT to check whether linearity of spatial summation depended on the colour of the screen.

Cells were classified as X or Y by means of their response to sine gratings, patterns in which the luminance varied sinusoidally with position in one dimension. The contrast of the gratings was reversed with a sinusoidal time course^{4,8,9}. A cell was called X if it responded to low and high spatial frequency patterns as if it received input from a single linear receptive field mechanism. This means that an X cell would respond to contrast reversal of the grating at the temporal modulation frequency, and its response would exhibit a sinusoidal dependence on the position (or spatial phase) of the grating^{4,8,9}. A cell was called Y if it responded to low spatial frequencies approximately like an X cell, but revealed only nonlinear behaviour at higher spatial frequencies. The nonlinear behaviour looked for, based on our experience with cat Y cells, was frequency doubling of responses to contrast reversal. In monkey Y cells, as in the cat, a frequency-doubled response was present at all positions of the grating. Classification of cells as X or Y did not depend on the colour of the screen in the experiments with Wratten filters. That retinal ganglion cells in the monkey can be classified as X or Y has been previously reported¹⁴. Thus, in the monkey, as in the cat, retinal ganglion cells confer their characteristic properties on their geniculate targets.

Cells classified as X by above criterion were found in all laminae of the macaque's LGN. Figure 1 shows the responses of a magnocellular X cell to the stimulus of a 1.5 cycle deg^{-1} sine grating undergoing contrast reversal with a sinusoidal time course at 2 Hz. Responses at three different positions or spatial phases are shown: at 0° spatial phase there was a maximal response, at 90° no response, and at 180° the response peaked again.

Almost all the parvocellular cells recorded were X cells (225 out of 226 cells recorded and assigned to the parvocellular laminae by histological reconstruction of tracks); only one was a Y cell. Most of the magnocellular cells recorded were also X cells (59 out of 77 cells assigned to the magnocellular layers); the rest were Y cells. Thus, our results show that magnocellular cells do not form a homogeneous class with respect to spatial summation properties. Similar results have been reported in preliminary form elsewhere¹⁵⁻¹⁸.

We next investigated functional differences between parvocellular and magnocellular cells by comparing their contrast sensitivities. Contrast sensitivity was determined as the reciprocal of the contrast required to give a modulated discharge with an amplitude of 5 impulses per second in response to a drifting sine grating. The spatial frequency of the grating was varied from 0 to 10 cycle deg^{-1} , and the peak contrast sensitivity for each cell was determined. The parvocellular cells had much lower peak contrast sensitivity than the magnocellular cells. The parvocellular cells had a peak contrast sensitivity of 10. Both X and Y cells in the magnocellular laminae had a peak contrast sensitivity of approximately 75. The contrast sensitivity of magnocellular cells was high enough that it might account for the behavioural contrast sensitivity of *Macaca fascicularis*¹⁹. Figure 2 shows that the magnocellular layers are the site of high contrast sensitivity and the parvocellular laminae contain cells which are relatively insensitive to luminance contrast. Our discovery that parvocellular neurones have low contrast sensitivity is consistent with previous evidence that these cells analyse colour^{10,12,20,21}. Note that peak contrast sensitivity did not show a strong dependence on the colour of the CRT screen in the experiments with Wratten filters.

The contrast sensitivities of cells in the magnocellular laminae of the monkey are comparable with those of cells in the A and A1 laminae of the cat LGN. As the parvocellular X cells were far less sensitive to contrast, the proposed homology between the X cells in the parvocellular laminae of the monkey and X cells in the cat LGN¹⁰⁻¹² is not compelling. A closer homology exists between the X and Y cells of the magnocellular laminae and the X and Y cells of the cat LGN. The parvocellular laminae of the monkey's LGN, and their retinal inputs, seem to form a visual neural pathway which is not present in cats.

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- Enroth-Cugell, C. & Robson, J. G. *J. Physiol., Lond.* **187**, 517-552 (1966).
- Rodick, R. W. A. *Rev. Neurosci.* **2**, 193-225 (1979).
- Lennie, P. *Vision Res.* **20**, 561-594 (1980).
- Hochstein, S. & Shapley, R. M. *J. Physiol., Lond.* **262**, 237-264 (1976).
- Shapley, R. M. & Hochstein, S. *Nature* **256**, 411-413 (1975).
- Cleland, B. G., Dubin, M. W. & Levick, W. R. *J. Physiol., Lond.* **217**, 473-496 (1971).
- Hoffmann, K. P., Stone, J. & Sherman, S. M. *J. Neurophysiol.* **35**, 518-51 (1972).
- So, Y. T. & Shapley, R. M. *Expl Brain Res.* **36**, 535-550 (1979).
- So, Y. T. & Shapley, R. M. *J. Neurophysiol.* **45**, 107-120 (1981).
- Dreher, B., Fukada, Y. & Rodick, R. W. *J. Physiol., Lond.* **258**, 433-452 (1976).
- Sherman, S. M., Wilson, J. R., Kaas, J. H. & Webb, S. V. *Science* **192**, 475-477 (1976).
- Schiller, P. H. & Malpeli, J. G. *J. Neurophysiol.* **41**, 788-797 (1978).
- Shapley, R. M. & Rossetto, M. *Behav. Res. Meth. Instrum.* **8**, 15-20 (1976).
- De Monasterio, F. *J. Neurophysiol.* **41**, 1394-1417 (1978).
- Kaplan, E. & Shapley, R. M. *Invest. Ophthalmol. vis. Sci. suppl.* **19**, 41 (1980).
- Shapley, R. M. & Kaplan, E. *Neurosci. Abstr.* **6**, 583 (1980).
- Blakemore, C. B. & Vital-Durand, R. *Trans. ophthalmol. Soc. U.K.* **99**, 363-368 (1980).
- Lennie, P. & Derrington, A. M. *Invest. Ophthalmol. vis. Sci. suppl.* **20**, 14 (1981).
- DeValois, R., Morgan, H. & Snodderly, D. M. *Vision Res.* **14**, 75-81 (1974).
- Wiesel, T. & Hubel, D. H. *J. Neurophysiol.* **29**, 1115-1156 (1966).
- DeValois, R. & DeValois, K. in *Handbook of Perception* Vol. 5, 116-168 (1975).

Two genes interact to control development of a lymphoid/erythroid hyperplastic disorder of mice

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We have previously established the presence of a gene in mice (called *Lus*)¹ which is dominant for the suppression of cytostasis, an experimental assay of cell-mediated immunity². Here we describe the definition of a gene (segregating independently of *Lus*) that, in combination with the dominant gene *Lus*^a, is homozygous recessive for a lymphoid hyperplastic disorder of T and B cells. We have called this gene *Arp*. The disease involves abnormalities of lymphopoietic and other organs, and could be a model system for regulatory defects of the immune system.

'Cytostasis' is an *in vivo/in vitro* assay of cell-mediated immunity³. In our experiments mice were sensitized by injections of allogeneic lymphoid cells⁴. After 14 days we tested lymph node cells from sensitized mice in an *in vitro* cytostasis assay, using tumour cells as target cells.

Cytostasis is assayed by the uptake of radioactive thymidine by the tumour cells and is positive if uptake is inhibited by the effector cells. Both T and B cells (A. M.-R., L. DeG. and H. F., in preparation) are capable of causing cytostasis. Allogeneic cytostasis following sensitization across the H-2 barrier usually gives a positive result. However, we noted that whereas immunization of CBA/H (H-2^k) mice with BALB/c spleen cells led to the production of cytostatic effectors against SL2 (H-2^d) tumour cells (a spontaneous Thy-1-positive DBA/2 lymphoma), immunization with B10.D2 (also H-2^d) spleen cells failed to generate anti-H-2^d effectors in the CBA/H (H-2^k) recipients. Mating B10.D2 with BALB/c mice produced F₁ hybrids whose spleen cells also did not stimulate the production of cytostatic effector cells, suggesting the presence of a dominant suppressor gene in the B10.D2 mice. This finding was further studied and confirmed using (B10.D2 × BALB/c)F₁ × BALB/c backcross mice up to the fourth generation² and beyond. Approximately 50% of backcross mice from each generation failed to stimulate the production of cytostatic effectors and were therefore carrying the cytostasis suppressor gene *Lus*^a (ref. 1 and Tables 1, 2). These were selected for further backcrossing to BALB/c, to develop a strain congenic with BALB/c at the suppressor locus. This was done up to the tenth generation backcross.

After the fourth backcross mating, we first noted the appearance of the hyperplastic disorder with abnormalities in the lymphopoietic and other organs in ~50% of 3-4-week old mice carrying the suppressor gene (Table 2), that is, cytostasis-negative mice. The disorder was manifested by gross hyperplasia of the thymus, spleen (Fig. 1), Peyer's patches and lymph nodes. There were concomitant histopathological changes in these

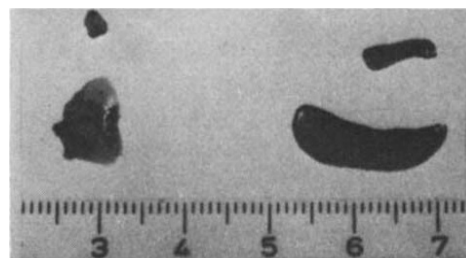


Fig. 1 Spleen and thymus from hyperplastic (right) and non-hyperplastic (left) cytostasis-negative littermates. Note the approximate 10-fold difference between the size of the organs.

Table 1 Data demonstrating the effects of the suppressor gene on the generation of cytostasis in CBA/H (H-2^k) mice

Immunizing Bc Mice	c.p.m. $\pm$ s.d. of SL2 (H-2 ^d) tumour targets cultured with:		% Inhibition of tumour growth
	Normal lymphocytes	Immune lymphocytes	
5th generation Cyts(+)	28,727 $\pm$ 2,618	10,383 $\pm$ 648	64
5th generation Cyts(-)	28,724 $\pm$ 2,618	28,828 $\pm$ 1,680	0
5th generation Cyts(-) with hyperplasia	28,724 $\pm$ 2,618	29,379 $\pm$ 1,131	0
6th generation Cyts(+)	56,582 $\pm$ 840	20,378 $\pm$ 811	64
6th generation Cyts(-)	56,582 $\pm$ 840	57,054 $\pm$ 3,188	0
6th generation Cyts(-) with hyperplasia	56,582 $\pm$ 840	64,123 $\pm$ 6,047	0
7th generation Cyts(+)	33,060 $\pm$ 1,472	17,558 $\pm$ 1,416	47
7th generation Cyts(-)	56,582 $\pm$ 840	54,665 $\pm$ 2,196	4
7th generation Cyts(-) with hyperplasia	56,582 $\pm$ 840	53,053 $\pm$ 2,728	7

CBA/H mice were immunized intraperitoneally with 10⁷ spleen cells obtained by biopsy from each backcross mouse, which was earmarked. 10–20 days after immunization, spleen cells were removed from the immunized mice under anaesthesia and cultured at a ratio of 10:1 with (SL2) tumour targets of DBA/2 origin in RPMI medium containing 5% fetal calf serum and antibiotics. Normal CBA/H lymphocytes were used as controls. The cultures were maintained for 48 h at 37 °C in a 4% CO₂, 10% N₂, 86% O₂ atmosphere. Tritiated thymidine was then added and the cultures were incubated for a further 16 h. The uptake of isotope, which was largely that of the tumour targets, was then measured and the inhibition of tumour growth calculated according to the following formula

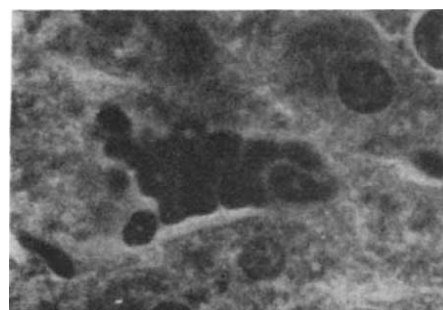
$$\% \text{ Inhibition} = 100 - \frac{\text{c.p.m. of tumour cells + immune lymphocytes}}{\text{c.p.m. of tumour cells + normal lymphocytes}} \times 100$$

Cyts(+) and Cyts(-) are, respectively, mice producing and failing to produce cytostatic effector cells.

tissues (Fig. 2a, b) as well as lymphocytic and metamyelocytic accumulations in the liver (Fig. 3) and hyperplasia of the bone marrow. There was marked general thymic hyperplasia and thymic medullary hypercellularity ('thymitis') in the T lymphoid system, accompanied by paracortical hyperplasia of the lymph nodes. Evidence of germinal centre development was also seen in spleens and lymph nodes of affected mice, and plasma cells were abundant in the lymph node medullae.

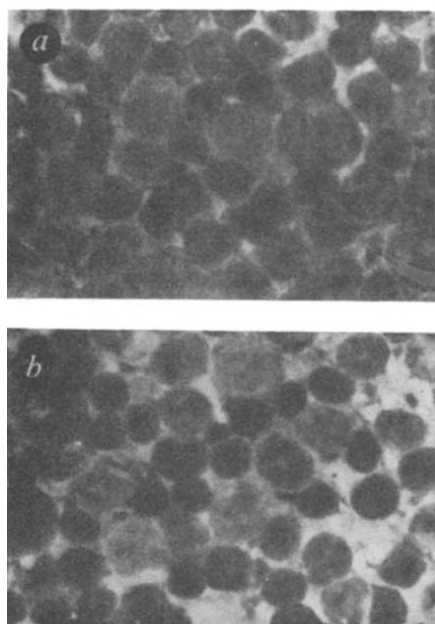
The hypercellularity of bone marrow and spleen was due to two components: haemopoiesis, both erythroid and myeloid, and enlargement of malpighian follicles with occasional germinal centre formation (spleen). The presence of excess stainable iron was particularly evident in spleens of mice affected with the disease. There was no evidence of lymphoid or haematopoietic malignancy.

These lesions therefore represent a dyshaematopoiesis associated with lymphoid hyperplasia of both T and B lymphoid components (T more marked than B). Aspects of this pathological picture are consistent with the disorder being due to a primary haemolytic process or autoimmune disorder—with

**Fig. 3** Section of liver showing focal infiltration with metamyelocytes.

secondary hyperplasia as in NZB mice⁵. Although this disease is debilitating, fatality in affected mice observed up to 6 months was not greater than in controls.

As this lymphoproliferative syndrome was found in approximately half of the cytostasis-negative mice (those with the *Lus*^a cytostasis suppressor allele) from all backcross generations (Table 2), the involvement of a second gene in the control of the disorder was suggested, and was designated *Arp*. The syndrome occurs only in *Lus*^a-positive mice and so presumably *Lus*^a interacts epistatically with *Arp*. Because the disease occurs in 50% of the *Lus*^a-positive mice, the genotypes of the diseased

**Fig. 2** Splenic impressions from hyperplastic and non-hyperplastic cytostasis-negative littermates. Note the presence of large numbers of blast cells with nucleoli (a) compared with predominantly mature leukocytes in the smears made from the non-hyperplastic spleens (b).**Table 2** Unigenic segregation of the cytostasis suppressor gene allele *Lus*^a in (B10.D2 $\times$ BALB/c)F₁ $\times$ BALB/c backcross mice

Backcross* generation	No. of mice tested	No. of Bc mice Cyts(+)	No. of Bc mice Cyts(-)	No. hyperplastic
1	8	3	5	NT
2	12	6	6	
3	18	9	9	
4	16	9	7	
5	50	21	29	14
6	46	22	24	10
7	20	9	11	6
8	21	10	10	5
9	17	9	8	4
10	12	6	6	3
Total	220	47%	53%	(47%)†

Segregation was assayed by the capacity of the backcross mouse (H-2^d) spleen cells to inhibit stimulation of cytostatic effectors in CBA/H recipients (H-2^k) against SL2 (H-2^d tumour target cells) and with the second gene (*Arp*) producing the hyperplastic disorder in Cyts(-) backcross mice. NT, not tested.

*The same BALB/c male was mated with (B10.D2 $\times$ BALB/c)F₁ females and four healthy Cyts(-) females selected from each backcross generation through to the tenth backcross.

†47% of Cyts(-) mice from the fifth and subsequent backcross generations presented a lymphohyperplastic syndrome described in the text.

Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted

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To produce antibody, bone-marrow-derived (B) lymphocytes generally require the synergistic action of a thymus-derived (T) lymphocyte, termed a helper T cell (Th)¹. The interaction of Th with B cells is antigen specific and requires that both the T and the B cell be specific for physically linked antigenic determinants². More recently it has been demonstrated in numerous systems that the interaction of Th and B cells is governed by antigens encoded within the major histocompatibility complex (MHC), such that Th and B cells must be identical in the I region of the MHC in order to cooperate efficiently³⁻⁵. This finding has been termed MHC restriction. However, studies using populations of T cells as a source of Th could be influenced by the presence of alloreactive T cells, suppressor T cells and other T-cells subsets, thus making interpretation difficult. Moreover, it has been proposed⁶ that MHC restriction governs not the interaction of Th with B cells, but rather the interaction of Th cells with antigen-presenting cells (APCs), whereafter the interaction of Th with B cells is said to be unrestricted. We have now attempted to clarify this situation by examining the interaction of soft agar colonies and clones of antigen-specific, MHC-restricted proliferating T cells^{7,8} with purified B cells using several different assays. We conclude that the antigen-specific cooperative interactions between Th and B cells are themselves MHC restricted, and that B cells bearing the wrong MHC-encoded antigens cannot be activated by addition of APCs matched to the Th cell but differing from the B cell at MHC.

As a source of Th cells, we have used BALB/c (H-2^d) and BALB.B (H-2^b) T cells primed with hen's egg albumin (OVA), cloned in soft agar and propagated *in vitro* according to Sredni *et al.*⁷. Evidence that these cells were antigen specific and MHC restricted in their proliferative responses is presented elsewhere⁸. The cells were then tested for their interaction with B cells in three different assays.

When antigen-specific, MHC-restricted T-cell colonies are treated with mitomycin C and added to B cells purified by elution from goat anti-mouse immunoglobulin (GaMIg) plates⁹ or by treatment of spleen cells with anti-Thy-1.2 antiserum and complement, intense B-cell proliferation ensues in the presence of specific antigen, peaking 48 h after initiation of the culture. This is not classically an assay for T-cell help, but seems rather to measure the polyclonal activation of B-cell proliferation by T cells. The data in Table 1 show that this response is antigen specific (furthermore, the response did not occur in the presence of 100 µg ml⁻¹ keyhole limpet haemocyanin), MHC restricted between T and B cells, and cannot be overcome by addition of mitomycin C-treated spleen cells as a source of APCs syngeneic to the Th but allogeneic to the B cells (Table 1b). Figure 1 shows a second experiment in which the Th colony cells are titrated. In this instance, some induction of allogeneic B-cell proliferation is seen in the presence of mitomycin C-treated syngeneic spleen cells, but it is much less than that observed when Th and B cells are MHC matched, and is relatively little influenced by Th-cell dose. Although allogeneic B cells can be stimulated to proliferate in this manner, the response is meagre at best and occurs only in some experiments. Thus, we conclude that Th cells will induce B-cell proliferation in the presence of antigen, and that this interaction is MHC restricted largely or entirely between the Th and B cells themselves.

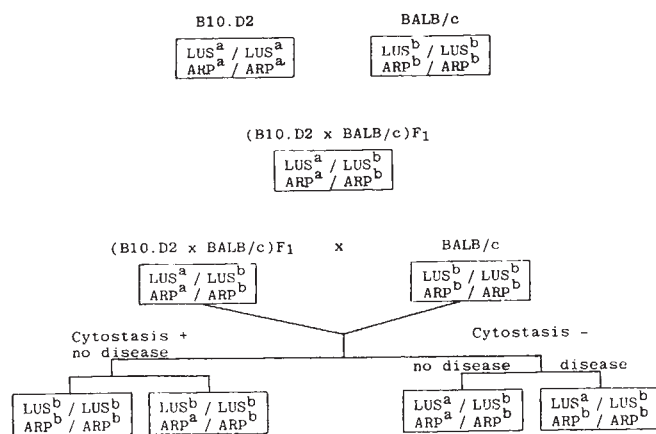


Fig. 4 Proposed genotype of B10.D2, BALB/c, (B10.D2 x BALB/c)F₁ and backcross to BALB/c mice, with respect to the allelic expression of *Lus^a* (suppressor) and *Arp^a* protector (or *Arp^b/Arp^b* recessive hyperplastic) genes. *Lus^a*: cytostasis suppressor (heterozygote co-dominant); *Lus^b*: cytostasis enhancer; *Arp^a*: protector against hyperplastic disorder (heterozygote co-dominant); *Arp^b*: inducer of hyperplastic disorder (recessive homozygote). Genotype *Lus^a/Lus^b* *Arp^b/Arp^b* suggests that epistasis of recessive *Arp^b/Arp^b* and co-dominant *Lus^a* is needed to produce the hyperplastic disorder, or that protector *Arp^a* is a co-dominant allele preventing the development of the hyperplastic disorder.

mice with respect to the second gene must be *Arp^b/Arp^b*, and *Lus^a* and *Arp* genes segregate independently. Figure 4 shows the segregation of these genes and the proposed phenotypes of the parental strains and the crosses. (The disease is produced when the *Arp^b* gene from BALB/c is homozygous and associated with *Lus^a*.) There is no evidence of linkage of these genes to sex chromosome genes (data not shown).

We found that the disease was present in several mice of the first generation backcross, but was missed in the early matings of the first backcrossing programme. If female cytostasis-negative mice were randomly selected for backcrossing, there would be a probability of 1/16 that after four generations *Arp^a* would still be segregating. However, these odds are increased because we selected for healthy mice (*Arp^a/Arp^b* heterozygotes) for backcrosses. Also, we selected for coloured coats, and *Arp^a* might be linked to a coat colour gene. When the disease appeared, only healthy non-hyperplastic mice that failed to stimulate the production of cytostatic effectors were then selected for further matings. Two genetic traits were thus simultaneously selected, one associated with *Lus^a* (cytostasis suppressor) and the other with *Arp^a* (a healthy mouse). This selection procedure allowed for segregation of these two genes in all successive backcross matings, resulting in the same proportional representation of sick and healthy mice carrying *Lus^a* at each future generation.

The way in which a genetic marker for a gene silent in its original context can, on transfer to a different genetic background, bring about a hyperplastic syndrome is intriguing. One can envisage that a human analogue of the suppressor gene may be transmitted to offspring with defective regulatory genes and thus promote neoplastic or hyperplastic cellular transformation.

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- DeGiorgi, L. thesis, London Univ. (1979).
- DeGiorgi, L., Matossian-Rogers, A. & Festenstein, H. *Immunology* **36**, 711-718 (1979).
- Chia, E. & Festenstein, H. *Eur. J. Immun.* **3**, 483-487 (1973).
- DeGiorgi, L., Biasi, G. & Festenstein, H. *J. Immunogenet.* **5**, 49 (1978).
- Holmes, M. C. & Burrell, F. M. *Ann. int. Med.* **59**, 265-273 (1963).

Table 1 Induction of B-cell proliferation by OVA-specific T-cell colonies and clones is antigen specific and MHC restricted

a, BALB/c B-cell proliferative response* (Δ c.p.m.) with colony† number:						
Antigen added	A3a	A4a	C5b	D2b	C1a	Normal BALB/c T cells
0	(7,700)‡	(9,500)	(4,400)	(7,200)	(3,800)	(3,400)
100 μ g ml ⁻¹ OVA	111,200	120,800	83,400	76,100	69,800	900
b, B-cell proliferative response to OVA (Δ c.p.m.) using B cells from:						
Cloned cells tested	BALB/c (H-2 ^d)	BALB-B (H-2 ^b)	BALB-B + BALB/c (mito)§	BALB-K (H-2 ^k)	BALB-K + BALB/c (mito)	
Cla	58,500 (17,400)	4,800 (22,000)	4,900	~1,900 (31,000)	3,000	
Cla	19,900 (2,000)	1,500 (4,300)	NT	3,200 (3,700)	NT	
CIG	63,400 (48,600)	NT	NT	5,400 (54,600)	NT	

* Normal T cells (the non-adherent fraction collected after incubation of spleen cells on goat anti-Mlg antibody-coated dishes⁹) or T-cell colonies were incubated (30 min at 37 °C) with mitomycin C (30 μ g ml⁻¹), which effectively blocked DNA synthesis, and $2-4 \times 10^4$ cells were added to 4×10^5 B cells prepared by adherence to goat anti-Mlg antibody-coated dishes⁹ (a, A3a, A4a, C5b, D2b and normal T cells; b, experiments of lines 2 and 3), or by treatment with anti-Thy 1.2 and complement (a, Cla; b, experiment of line 1). Cultures were pulsed with 1 μ Ci ³H-thymidine (60 Ci mmol⁻¹) after 48 h, and collected 3 h later.

† BALB/c OVA-specific T-cell colonies were prepared as described by Sredni *et al.*⁷ with the following modifications: T cells were prepared by passage over Ig-anti-Ig columns¹⁵, and the clones were stimulated with 5×10^5 mitomycin C-treated spleen cells every 5-7 days. Colonies were tested 2-3 months after cloning. By immunofluorescence tests all cloned cells were Thy1.2⁺, Lyt-1.2⁺, Lyt-2.2⁻.

‡ Background c.p.m. in the absence of antigen are shown in parentheses, and have been subtracted from responses. NT, not tested.

§ 2×10^5 mitomycin C-treated BALB/c spleen cells were added to the cultures.

|| Colony Cla was grown in bulk culture on B10-G spleen stimulator cells plus 20% T-cell growth factor (TCGF) (v/v) in the absence of OVA or H-2^d stimulators for 3 weeks, and then recloned by limiting dilution on BALB/c mitomycin C-treated spleen cells with 20% TCGF and OVA (200 μ g ml⁻¹). After 2-3 weeks six subclones were isolated, of which CIGI is an example.

Table 2 Induction of B-cell proliferation by normal T cells or T-cell colonies and clones mediated by rabbit anti-mouse brain antiserum

a, B-cell proliferative response* (Δ c.p.m.) with colony number:						
Serum added	A3a	A4a	C5b	D2b	C1a	Normal BALB/c T cells
NRS	(7,700)†	(9,500)	(4,400)	(7,200)	(3,800)	(2,900)
RaMBr	70,600	122,400	110,000	116,500	24,100	45,000
b, B-cell proliferative response (Δ c.p.m.) using B cells from:						
Cloned cells tested:	BALB/c (H-2 ^d)	BALB-B (H-2 ^b)	BALB-K (H-2 ^k)			
Cla	16,200 (2,000)	50,200 (4,300)	13,900 (3,700)			
CIGI	83,800 (31,900)	NT	38,400 (42,000)			

Colony preparation was as described in Table 1 legend.

* Mitomycin C-treated T cells were incubated with B cells and pulsed with ³H-thymidine after 48 h as described in Table 1 legend, but in these experiments, instead of OVA, normal rabbit serum (NRS) or rabbit anti-mouse brain (RaMBr) antiserum absorbed with mouse liver ($\times 3$) were added to the cultures at a final dilution of 1:100.

† Background c.p.m. with NRS are shown in parentheses and have been subtracted from the responses with RaMBr to calculate Δ c.p.m.

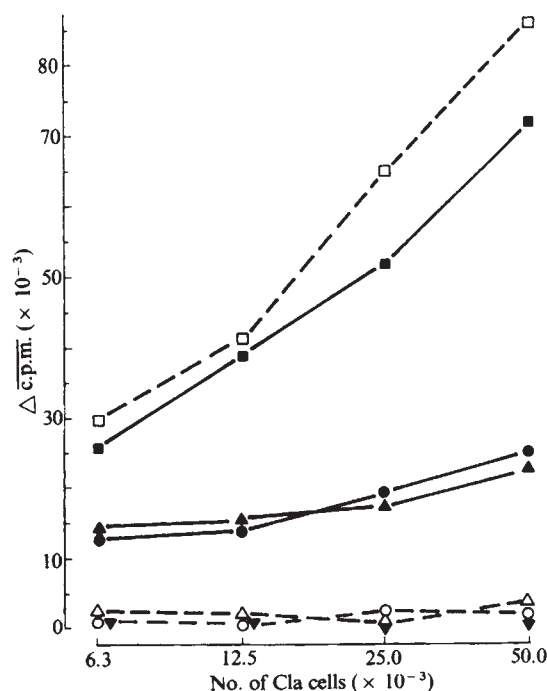


Fig. 1 BALB/c T_{OVA} colony cells (Cla) were treated with mitomycin C and titrated for their ability to induce proliferation in purified, non-immune, spleen B cells either in the absence (open symbols ---) or presence (closed symbols —) of 2×10^5 mitomycin C-treated BALB/c spleen cells added with OVA (100 μ g ml⁻¹) as a supply of syngeneic antigen-presenting cells. 4×10^5 B cells of the following strains were tested: BALB/c (□), BALB/K (○) or BALB/B (Δ). A control in the absence of B cells was performed (▼). After 48 h the cultures were pulsed with ³H-thymidine and the results are expressed as Δ c.p.m., the difference between the response stimulated by OVA and controls in the absence of antigen.

Two objections could be raised to these experiments. The first is that the Th cells might in some way be suppressing the response of allogeneic B cells, and the second is that this response does not represent help, which involves both B-cell proliferation and differentiation to antibody-forming cells¹⁰. We have therefore performed two further sets of studies to deal with these potential objections. In the first, Th- to B-cell interactions were initiated not with antigen but with rabbit anti-mouse brain antiserum (RaMBr), which activates T-cell proliferation in the presence of B cells in a non-MHC-restricted fashion¹¹. We have subsequently shown that it can also induce B-cell proliferation and polyclonal antibody production 48 h after initiation of culture in the presence of mitomycin C-treated Lyt-1⁺, 2⁻ T cells. Because the T-cell colonies we have prepared are surface Ig⁺, Thy-1⁺, Lyt-1⁺, 2⁻, we treated them with mitomycin C and added them to purified B cells in the presence of normal rabbit serum (NRS) or RaMBr. Both normal BALB/c T cells and antigen-specific, MHC-restricted T-cell colonies induce a potent, polyclonal B-cell proliferative response in the presence of RaMBr but not NRS (Table 2), and this response is both T-cell dependent and unaffected by the MHC genotype of the responding B cell (Table 2b). Thus, these studies demonstrate that both normal T cells and MHC-restricted T-cell colonies can activate allogeneic B cells via RaMBr. It therefore seems unlikely that the failure of allogeneic B cells to respond to antigen in the presence of cloned T cells is due to some inhibitory effect of the T cells, but rather that it represents MHC-restricted activation of B cells by T cells.

To examine this question further, the ability of B cells to be induced to produce specific antibody by such Th cells was examined in an *in vitro* response to the hapten phosphorylcholine (PC) coupled to OVA, a system previously used by Bottomly and Jones¹² to explore the regulation of idiotype expression. In the present experiments, two colonies, one derived from BALB/c (H-2^d) and the other from the MHC-

congenetic line BALB-B (H-2^b), were tested for their ability to help BALB/c or C57BL/6J (H-2^b) B cells. Furthermore, to test critically whether Th to B interactions were MHC restricted, we mixed purified B cells from the two strains with T-cell colonies of one type, cultured for 5 days with antigen, and assayed the plaque-forming cells (PFCs) before and after treatment with a monoclonal anti-H-2.5 antibody and rabbit complement. H-2.5 is present on H-2^b but not H-2^d cells, and thus the genotype of each PFC could be determined. If restriction operates at the level of Th to APC, and the genotype of the B cell is irrelevant as has been proposed⁶, then in these mixtures half of the PFCs should be H-2.5 positive and half negative, independent of the MHC restriction of the Th cells. In fact, although there is some nonspecific killing of PFCs by the antibody, as shown in the syngeneic mixtures, it is clear that there is little or no cooperation between Th cells and non-self B cells in this experiment (Table 3). One cannot argue, in this instance, that we have not added the necessary APCs, as a perfectly good PFC response is obtained in both cases. This experiment thus demonstrates conclusively that the MHC-restricted interaction in cooperation between Th and B cells in the response to soluble hapten-carrier conjugates occurs between Th and the B cells themselves, and does not involve non-MHC-restricted activation of B cells by Th cells via APCs.

B cells bear large amounts of Ia antigens on their surfaces¹³. The interaction of Th with B cells is known to be restricted by Ia antigens^{3,14}. We interpret the present studies as showing that the function of these Ia molecules is to promote the interaction of Th cells with their appropriate target cells, the B cells. APCs also have Ia on their surface, and it is clear that Th-like cells can also interact with antigen in the context of APC Ia. Perhaps APCs, being non-antigen specific, are especially important for the initial activation of Th cells, after which Th cells preferentially interact with B cells bearing both the specific antigen bound by the B cell's immunoglobulin receptor and the appropriate Ia antigen. Thus, MHC restriction is seen as providing the Th cell with a mechanism of finding antigen only on the surface of those cells with which it can functionally interact, namely, B cells and APCs. If Th- to B-cell interactions were not themselves MHC restricted, this interpretation would not make sense. However, the present studies show that this interaction is indeed MHC restricted between Th and B cells.

The present report also describes two convenient and rapid methods for measuring Th- to B-cell interactions in proliferation assays. The polyclonal B-cell response to antigen in the presence of both Th colonies and cloned Th is not fully understood. Because it requires 1,000 times as much antigen as the *in vitro* specific anti-PC PFC response to PC-OVA, it may be that all B cells acquire a small amount of antigen at high antigen dose, independent of their immunoglobulin receptor, and thus can be

induced to respond by the Th cells. If this is the case, it might imply that the immunoglobulin receptor is not involved in this form of B-cell proliferation. We are now investigating the role of the immunoglobulin receptor in this response, and also whether such cells subsequently secrete immunoglobulin. The assay involving RaMBR is reported in detail elsewhere. We foresee a role for this assay in assessing T- and B-cell function in man, as RaMBR-mediated T to B interactions do indeed lead to immunoglobulin production (J. P. Tite and B.J., unpublished), and because the interaction is not MHC restricted, the assay could be used between any two individuals.

We conclude that the interaction of Th cells with B cells is antigen specific and is itself MHC restricted. This conclusion supports the notion that Ia on B cells is critically important in promoting appropriate interactions with Ia-restricted helper T cells.

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1. Mitchell, G. F. & Miller, J. F. A. P. *J. exp. Med.* **128**, 821-837 (1968).
2. Mitchison, N. A. *Eur. J. Immun.* **1**, 18-27 (1971).
3. Katz, D. W., Graves, M., Dorf, M. E., DiMuzio, H. & Benacerraf, B. *J. exp. Med.* **141**, 263-268 (1975).
4. Sprent, J. *Immun. Rev.* **42**, 108-137 (1978).
5. Schreier, M. H. & Tees, R. *Int. Archs Allergy appl. Immun.* **61**, 227-237 (1980).
6. Singer, A., Hathcock, K. S. & Hodes, R. J. *J. exp. Med.* **149**, 1208-1226 (1979).
7. Sredni, B., Tse, H. Y. & Schwartz, R. H. *Nature* **283**, 581-583 (1980).
8. Janeway, C. A. & Conrad, P. J. *Behring Inst. Mitt.* (submitted).
9. Wysocki, L. J. & Sato, V. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2844-2848 (1978).
10. Schimpl, A. & Wecker, E. *Transplant Rev.* **23**, 176-188 (1975).
11. Jones, B., Dockrell, H. & Janeway, C. A. in *T and B Lymphocytes: Recognition and Function* (eds Bach, F. H., Bonavida, B., Vittieta, E. S. & Fox, F. C.) 175-180 (Academic, New York, 1979).
12. Bottomly, K. & Jones, F. in *2nd Int. Conf. B Lymphocytes in the Immune Response* (eds Klinman, N., Mosier, D. E., Scher, I. & Vittieta, E.) 415 (Elsevier, Amsterdam, 1981).
13. Sachs, D. H. & Cone, J. L. *J. exp. Med.* **138**, 1289-1304 (1973).
14. Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H. & Benacerraf, B. *J. exp. Med.* **138**, 734-739 (1973).
15. Wiggall, H. in *In vitro Methods in Cell Mediated and Tumour Immunity* (eds Bloom, B. R. & David, J. R.) 245-253 (Academic, New York, 1976).

Distinct genes for fibroblast and serum C1q

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Complement component C1 is composed of three subcomponents, C1q, C1r and C1s (refs 1, 2). The problem of which cells produce C1 *in vivo* has been controversial, and many cell types³⁻⁹ have been reported to synthesize these molecules. Material antigenically similar to C1q was reported¹⁰ to be synthesized and secreted by human and rat fibroblast cell lines. Reid and Solomon¹¹ subsequently showed that C1 haemolytic activity was secreted into the medium of cultured human fibroblast cell lines, and that the form of the C1q was unusual in that it had an apparently higher molecular weight than did C1q isolated from normal serum. As fibroblasts are an abundant cell type, this observation suggested that they might be a major contributor to serum C1q, secreting C1q precursor, pro-C1q. We describe here a genetic defect of serum C1q. Homozygotes for this defect do not produce functional plasma C1q, although they seem to produce normal fibroblast C1q. Heterozygotes produce both normal and defective serum C1q. This suggests that different genes code for the chains of C1q produced by fibroblasts and for those produced in serum.

Table 3 Cooperation between OVA-specific T-cell colonies and B cells is MHC restricted in the response to PC-OVA

B cells	Treatment to PFCs	Anti-PC-PFCs per 10 ⁶ B cells with Th colony		
		0	II 2 B6d (H-2 ^d)	II 2 D4b (H-2 ^b)
BALB/c (H-2 ^d)	0	3	112	10
BALB/c	Y-3+C	—	75 (33%)	—
C57BL/6 (H-2 ^b)	0	1	8	106
C57BL/6	Y-3+C	—	—	2 (98%)
BALB/c+C57BL/6	0	—	153	191
BALB/c+C57BL/6	Y-3+C	—	102 (33%)	18 (91%)

B cells were normal spleen cells treated with anti-Thy-1.2+C followed by anti-Lyt-1.2+ anti-Lyt-2.2 plus C. 3×10^6 viable cells placed in 1 ml culture with $0.1 \mu\text{g ml}^{-1}$ PC-OVA and 10^5 helper T cells. Five days later, plaque-forming cells (PFCs) were evaluated on PC-coupled sheep red blood cells. Values represent means of triplicate cultures. After culture, cells were treated with rabbit complement, or with monoclonal anti-H-2.5 antibody Y-3, which was raised in a BALB/c anti-H-2^b immunization and kills 100% of H-2^b spleen cells to a titre of $1:10^6$, but does not react with H-2^d. Helper T-cell colonies from BALB/c or BALB-B mice were prepared as described in Table 1 legend. Numbers in parentheses give per cent reduction in PFC response after treatment with anti-H-2^b monoclonal antibody and complement.

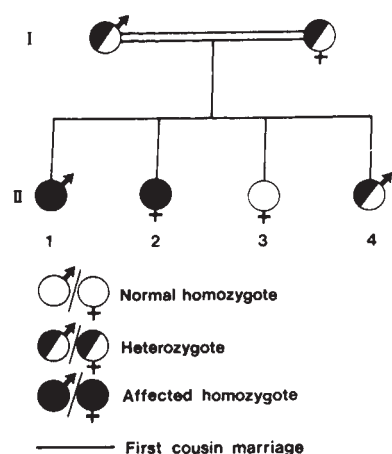


Fig. 1 Family tree showing the inheritance of the gene responsible for the production of the non-functional serum C1q. This condition appears to behave as an autosomal recessive in which both affected siblings, II 1 and II 2, are homozygous for the defective gene, and both parents and sibling II 4 are heterozygous. Skin biopsies were obtained from II 1 (SSF) and II 2 (RKF).

A family has been described¹² in which two siblings are totally deficient in serum C1q activity. The eldest child, a boy, suffers from a lupus-like syndrome and glomerulonephritis. The younger sister shows no evidence of nephritis clinically, but has cutaneous vasculitic lesions of the face and hands. Both children have a non-functional, but immunologically cross-reacting serum C1q. The normal and defective C1q in the family can be distinguished on Ouchterlony double-diffusion gels¹². The defective serum C1q is slightly less mobile on SDS-polyacrylamide gel electrophoresis (PAGE) in non-reducing conditions than is normal C1q (K.B.M.R., unpublished results), as shown in Fig. 3a. In addition, the patients' serum C1q behaves anomalously compared with normal C1q on a Sephadex G-200 column, and can be separated in non-dissociating conditions. Of two other siblings, one is entirely normal, and the other, apparently heterozygous, has both functional and non-functional C1q. The parents are first cousins, and they also have functional and non-functional C1q. Figure 1 shows the family tree.

To determine whether the fibroblasts of these patients produce the haemolytically inactive C1q that is found in their serum, we established skin fibroblast cultures, designated SSF (male) and RKF (female), from each of the two affected homozygous siblings. Human fibroblasts, designated Bu, from a C1q normal individual were used as the control culture. Cells were grown for 7 days in RPMI 1640 medium in the presence of 10% inactivated fetal calf serum (FCS), and the samples from days 0 and 7 compared. The FCS was inactivated by heating at 56 °C for 45 min, which destroys most, but not all, of the bovine C1 haemolytic activity. As seen in Fig. 2, SSF and RKF cultures gave the same titres of C1 haemolytic activity as did the control fibroblasts. Assay conditions indicated that these fibroblasts were producing C1r and C1s, in addition to active C1q, a finding consistent with previous work¹¹.

To characterize the structure of the fibroblast C1q produced by the serum C1q-deficient patients, immunoprecipitates derived from the supernatants of labelled cultures of SSF, RKF and Bu were compared on SDS-PAGE (Fig. 3a, b). These cultures were also assayed for haemolytic activity and found to be positive.

Cold carrier serum C1q was added to each sample of labelled fibroblast medium before immunoprecipitation. The immunoprecipitates were run on SDS-PAGE in reducing and non-reducing conditions. Figure 3a shows the positions of the cold carrier serum C1q, after protein staining. The reduced sample (lane 1) shows the three chains of serum C1q, A, B and C, at

apparent molecular weights 34,000, 31,000 and 27,000 (refs 13, 14), and the heavy and light chains of the rabbit immunoglobulin. The non-reduced sample (lane 2) shows the A-B and C-C dimers of C1q and the intact rabbit immunoglobulin. Figure 3b shows an autoradiograph of the whole gel. As found previously with fibroblast cultures¹¹, the larger-molecular-weight C1q does not enter the gel in the non-reduced form (lanes 5-7). When reduced, the radioactively labelled C1q immunoprecipitates from the three fibroblast cultures (lanes 2-4) all show the same two bands, with apparent molecular weights of 47,000 and 42,000. These same two bands were previously shown to be synthesized by fibroblasts¹¹. Both reduced and non-reduced samples show radioactive bands near the top of the gel. The identity of these bands is not certain, but they are probably fibronectin which could bind to the collagenous part of the cold carrier C1q, present in the immune aggregates, in a similar manner to the binding of fibronectin to denatured collagen (see, for example, ref. 15).

The results clearly indicate that the C1q secreted into the medium of the fibroblast cultures, derived from the two serum C1q-deficient siblings, has haemolytic activity comparable with that of the control culture. The radiolabelled immunoprecipitates from the culture medium also show no apparent differences between the patients and the control.

If fibroblasts were a major source of serum C1q, patients with a non-functional circulating C1q might be defective in a gene

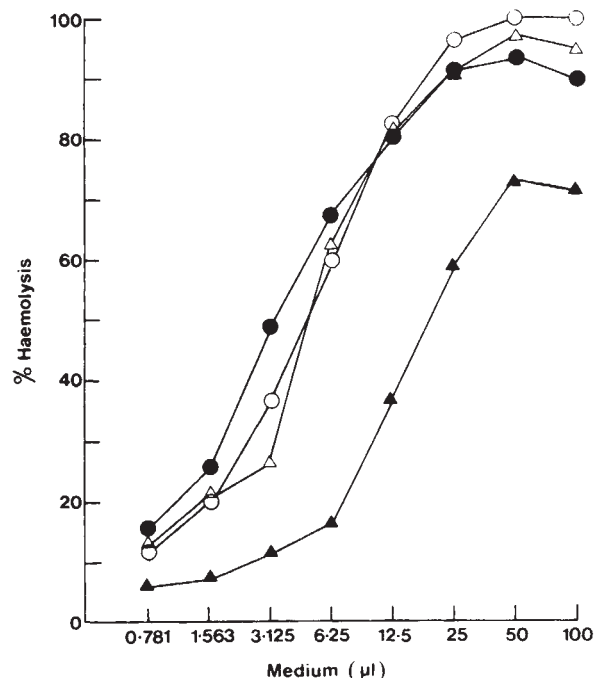


Fig. 2 Assay of component C1 haemolytic activity of day 0 control, and day 7 medium collected from fibroblast cultures of Butler (Bu), RKF and SSF. Preparation of the buffers and reagents used in the haemolytic assays was performed as described by Reid *et al.*¹⁶. Aliquots (100 μl) of doubling dilutions of the cell culture media were incubated with 100 μl of EAC4 cells (1×10^8 cells ml^{-1}) at 30 °C for 20 min, and then spun down at 2,000 r.p.m. for 10 min. The cell pellet was resuspended in assay buffer and incubated at 30 °C for 40 min, then component C2 (0.1 ml, 200 effective mol ml^{-1}) was added and the mixture incubated at 30 °C for 15 min. 0.1 ml of EDTA buffer, pH 7.4, was added and finally 0.2 ml of guinea pig serum, diluted 1/30 with isotonic buffer, pH 7.4, containing EDTA, was added and incubated at 37 °C for 30 min. After addition of 1.0 ml 0.15 M NaCl, the mixture was spun at 2,000 r.p.m. for 10 min and the degree of lysis determined by reading the A_{412} of the supernatant. ○, Bu; △, RKF (female); ●, SSF (male); ▲, day 0 control.

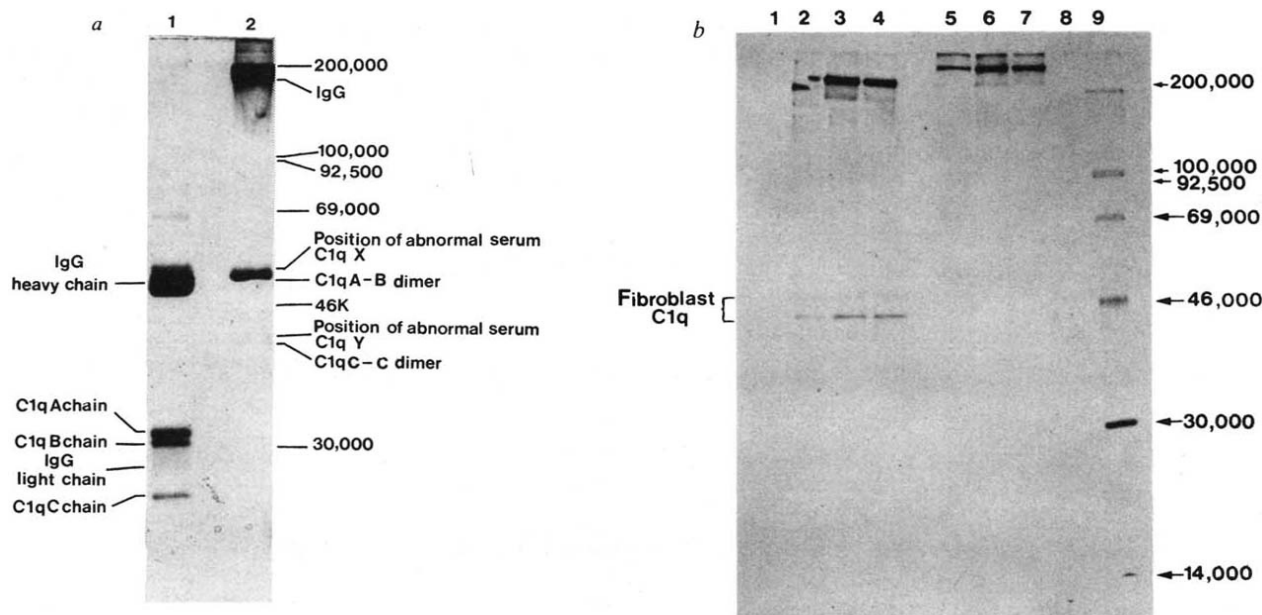


Fig. 3 *a*, The three fibroblast cell lines, Butler, RKF and SSF, were labelled over 72 h with 100 μCi ^{35}S -methionine, 16.3 μCi ^{14}C -proline and 16.3 μCi ^{14}C -glycine per flask of 2×10^6 cells. The radioactive amino acids were added directly to the medium containing inactivated FCS. A solution of carrier subcomponent C1q, prepared according to Reid¹⁷ (30 μl of 3.8 mg ml^{-1}), was added to the 30 ml culture medium collected from the three fibroblast cell lines. The medium was made 10 mM with respect to EDTA, and then concentrated to ~4 ml in an Amicon Diaflo 50-ml cell using a PM10 membrane. The concentrate was mixed with enough heat-inactivated rabbit anti-human serum subcomponent C1q to precipitate all the unlabelled carrier subcomponent C1q added. The rabbit anti-C1q was prepared according to Reid *et al.*¹³. The concentrate plus antiserum was incubated at 37 °C for 2 h, and then at 4 °C overnight. The immunoprecipitate formed was washed five times with 0.3 ml of 10 mM EDTA/150 mM NaCl, pH 7.4. After the final wash the samples were electrophoresed on a polyacrylamide gel in the presence of SDS as described by Fairbanks *et al.*¹⁸. The gel was stained for 1 h and then destained overnight. Lane 1, cold reduced serum C1q; lane 2, non-reduced cold serum C1q. Molecular weight markers (^{14}C -methylated protein mixture CFA.610, Radiochemical Centre) were myosin (MW 200,000), phosphorylase *b* (100,000 and 92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000) and lysozyme (14,300). Standards were run in reducing conditions. The mobility of the defective serum C1q in non-reducing conditions is indicated on this gel. This was determined in a parallel experiment (K.B.M.R., unpublished results). X and Y correspond to what are thought to be the A-B and C-C dimers of the abnormal serum C1q. *b*, The gel was impregnated with diphenyloxazole in dimethyl sulphoxide by the method of Bonner and Laskey¹⁹, dried and exposed to RP Royal X-Omat film at -70 °C for 2 weeks. Lane 2, reduced Bu; 3, reduced RKF; 4, reduced SSF; 5, non-reduced Bu; 6, non-reduced RKF; 7, non-reduced SSF.

coding for a processing enzyme of fibroblast C1q. In this event, one could not expect the heterozygote parents and sibling to have normal as well as abnormal circulating C1q, as co-dominant expression is not expected with a defect in a processing enzyme. Thus, these results strongly suggest that a cell type other than fibroblasts is responsible for the synthesis of serum C1q, and that there are distinct genes for the fibroblast C1q and serum C1q. The products of these genes would be two antigenically and physicochemically different C1q molecules.

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Dispermic origin of XY hydatidiform moles

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Complete hydatidiform mole is an abnormal human pregnancy with grossly swollen chorionic villi, usually with a 46,XX karyotype, and with a propensity to malignancy¹. The XX moles originate from fertilization of an 'empty egg' (resulting from either enucleation or inactivation of the female pronucleus) by a haploid sperm and its subsequent duplication^{2,3}, a process called diploid androgenesis. XY moles, although infrequent, are of special interest because their origin must differ from that of the XX moles. Two XY moles have been studied for their origin, but the results were inconclusive^{3,4}. We describe here a study of four XY moles, and provide evidence that they result from the fertilization of an empty egg by two haploid spermatozoa.

The macroscopic and microscopic findings of the four XY moles were indistinguishable from those of XX moles. The karyotypes of cells cultured from the molar tissue of the four patients were 46,XY. Q- and R-band chromosome heteromorphisms², histocompatibility leukocyte antigen (HLA) specificities, phosphoglucosyltransferase 1 (PGM₁) and esterase D

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1. Lepow, I. H., Naff, E. W., Todd, E. W., Pensky, J. & Hinz, C. F. *J. exp. Med.* **117**, 938-1008 (1963).
2. Gigli, I., Porter, R. R. & Sim, R. B. *Biochem. J.* **157**, 541-548 (1976).
3. Stecher, V. J., Morris, J. H. & Thorbecke, G. J. *Proc. Soc. exp. Biol. Med.* **124**, 433-438 (1967).
4. Day, N. K., Gewurz, H., Pickering, R. J. & Good, R. A. *J. Immun.* **104**, 1316-1319 (1970).
5. Muller, W., Hanasuske-Abel, H. & Loos, M. *J. Immun.* **121**, 1578-1584 (1978).
6. Colten, H. R., Borsos, T. & Rapp, H. J. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1158-1163 (1966).
7. Colten, H. R., Gorden, J. M., Rapp, H. J. & Borsos, T. *J. Immun.* **100**, 788-792 (1968).
8. Bing, D. H., Spurlock, S. E. & Bern, M. M. *Clin. Immun. Immunopath.* **4**, 341-351 (1975).
9. Morris, K. M., Colten, H. R. & Bing, D. H. *J. exp. Med.* **148**, 1007-1019 (1978).
10. Al-Adnani, M. S. & McGee, J. O'D. *Nature* **263**, 145-146 (1976).
11. Reid, K. B. M. & Solomon, E. *Biochem. J.* **167**, 647-660 (1977).
12. Thompson, R. A. *et al.* *New Engl. J. Med.* **303**, 22-24 (1980).
13. Reid, K. B. M., Lowe, D. M. & Porter, R. R. *Biochem. J.* **130**, 749-763 (1972).
14. Reid, K. B. M. & Porter, R. R. *Biochem. J.* **155**, 19-23 (1976).
15. Ruoslahti, E., Vaento, M. & Engrall, E. *Biochim. biophys. Acta* **534**, 210-218 (1978).
16. Reid, K. B. M., Sim, R. B. & Faiers, A. P. *Biochem. J.* **161**, 239-245 (1977).
17. Reid, K. B. M. *Biochem. J.* **141**, 189-203 (1974).
18. Fairbanks, G., Stech, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606-2617 (1971).
19. Bonner, M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).

Table 1 Mode of inheritance of heteromorphic features in XY moles

			Chromosomal heteromorphisms†								HLA		Enzyme variants		
			Age*	Karyotype	3	4	13	14	15	21	22	A	B	PGM ₁	ES-D
A	{	Mat	23 yr	46, XX	ab	aa	aa	ab	ab	aa	ab	2, 9	w51, w52	2-1	2-1
		Pat	25 yr	46, XY	aa	aa	aa	aa	bc	ab	ab	9, 11	40, w21	1-1	2-1
		Mole	67 days	46, XY	aa	aa	aa	aa	cc	ab	ab	9, 9	40, 40	1-1	2-2
B	{	Mat	27 yr	46, XX	aa	aa	aa	aa	aa	aa	ab	1, 2	40, 39	2-1	2-1
		Pat	27 yr	46, XY	aa	aa	ab	ab	bc	bc	aa	9, 11	7, 13	1-1	1-1
		Mole	65 days	46, XY	aa	aa	aa	aa	bc	bc	aa	11, 11	7, 7	1-1	1-1
C	{	Mat	23 yr	46, XX	aa	aa	ab	ab	ab	ab	aa	2, 2	w51, 40	1-1	2-1
		Pat	27 yr	46, XY	ab	aa	bc	aa	ac	ab	ab	2, 9	40, 40	2-1	2-1
		Mole	72 days	46, XY	ab	aa	bb	aa	cc	ab	ab	2, 2	40, 40	1-1	2-1
D	{	Mat	21 yr	46, XX	ab	aa	ab	ab	aa	ab	ab	—	—	1-1	1-1
		Pat	22 yr	46, XY	ac	aa	cd	ac	ab	bb	bc	—	—	1-1	1-1
		Mole	122 days	46, XY	cc	aa	cc	ac	ab	bb	cc	—	—	1-1	1-1

* Age at expulsion (days after last menstrual period in hydatidiform mole).

† a, b, c and d are symbols and do not represent specific heteromorphisms. —, Neither of maternal markers transmitted to the mole, indicating its androgenetic origin. □, A paternal marker transmitted in duplicate to the mole. □□, Both of paternal markers transmitted to the mole. A, B, C, D refer to the four moles studied.

(ES-D) genotypes were studied in the four XY moles and their parents. HLA-A and -B specificities were studied using peripheral blood lymphocytes from the parents and cultured molar fibroblasts by the standard National Institutes of Health two-stage microcytotoxicity test⁵. PGM₁ and ES-D genotyping was carried out on parental red cells and on uncultured molar villi with starch gel electrophoresis^{6,7}. These genetic markers were reliable in our hands in the study of the origin of XX moles (ref. 2 and unpublished data). The chromosomal markers are situated close to the centromeres and thus are usually free from meiotic crossing-over, while HLA and enzyme gene loci are situated relatively far from the centromeres, and thus may occasionally be subject to cross-over and resulting recombination.

The results of these studies are shown in Table 1. Mole A inherited both chromosomes 15 and HLA-B specificities exclusively from the father. Similarly, mole B showed an exclusively paternal inheritance of both chromosomes 15 and 21, as well as HLA-A and -B specificities. Mole C inherited both chromosomes 15 from the father. Mole D inherited pairs 3, 13 and 22 exclusively from the father. Thus, all four moles were androgenetic in origin. Further, each of the four moles had both homozygous (a paternal marker transmitted to the mole in duplicate) and heterozygous (both paternal markers transmitted to the mole) genetic markers. The fact that both homozygous and heterozygous chromosomal heteromorphisms were observed in each of the four moles indicates that the moles resulted from dispermy, that is, entry of an X and Y sperm into an 'empty egg'. Entry of a diploid spermatozoon resulting from tetraploid spermatogonia cannot be ruled out but is less likely. The results of the HLA studies do not support the cytogenetic findings, but this could well be a chance occurrence (one in eight).

We are aware of 121 complete moles karyotyped since 1977: 80 by us and 41 in the literature^{3,4,8-10}. This, excluding an isolated case report⁴, would give five XY moles out of 120 karyotyped, a rate of 4%. Dispermy would result in XX, XY and YY sex chromosome constitution, at a 1:2:1 ratio. YY moles are lethal, while XX dispermic moles are not. Therefore, some 2% of XX moles would also be the product of dispermy, a variety yet to be identified.

Mole B in our series is worthy of comment. The human chorionic gonadotropin (HCG) level in the mother remained high after expulsion of the mole, and roentgenography 8 weeks after expulsion revealed in the right lung a 15 × 13 mm rounded shadow not seen before. Chemotherapy was started and the urinary HCG level fell to normal in 10 weeks, while the pulmonary shadow remained unchanged 18 months after treatment. We proposed that the propensity to malignancy of complete moles is associated with single chromosomal mutation

that is of necessity homozygous in the 46, XX variety². This explanation might only apply to the XY dispermic moles, in which each spermatozoon would contribute the same chromosomal segment carrying the causative mutation. It would be interesting to know whether such a propensity is more prevalent in one of the two classes of complete mole¹¹.

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1. Vassiliakos, P., Rionton, G. & Kajii, T. *Am. J. Obstet. Gynec.* **127**, 167-170 (1977).
2. Kajii, T. & Ohama, K. *Nature* **268**, 633-634 (1977).
3. Jacobs, P. A., Wilson, C. M., Sprengle, J. A., Rosenheim, N. B. & Migeon, B. R. *Nature* **286**, 714-716 (1980).
4. Surti, U., Szulman, A. E. & O'Brien, S. *Hum. Genet.* **51**, 153-156 (1979).
5. Ray, J. G., Hare, D. B., Peterson, P. D. & Kaybee, D. E. (eds) *Manual of Tissue Typing Techniques*, 20-22 (NIH, Bethesda, 1974).
6. Spencer, N., Hopkinson, D. A. & Harris, H. *Nature* **204**, 742-743 (1964).
7. Hopkinson, D. A., Mestriner, M. A. & Cortner, J. *Ann. hum. Genet.* **37**, 119-137 (1973).
8. Wake, N., Takagi, N. & Sasaki, M. *J. natn. Cancer Inst.* **60**, 51-57 (1978).
9. Wake, N., Shiina, Y. & Ichino, K. *Proc. Jap. Acad.* **54**, 533-537 (1978).
10. Lawler, S. D. *et al.*, *Lancet* **ii**, 580 (1979).
11. Kajii, T. *Gann Monogr. Cancer Res.* **25**, 189 (1980).

RNA is synthesized at the nuclear cage

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The photomicrographs of 'genes in action' taken by Miller and colleagues strikingly illustrate transcription of eukaryotic genes by RNA polymerase moving along the DNA^{1,2}. Although completed transcripts may be associated with sub-nuclear structures³⁻⁷, the view that a mobile polymerase processes along the DNA remains unchallenged. Here we examine an alternative view—that transcription occurs as DNA passes through a transcription complex fixed to a sub-nuclear structure, and we show that transcribed sequences are closely associated with a sub-nuclear structure that we call a nuclear cage.

When nuclei are isolated by conventional procedures, their DNA is invariably broken, mainly by endonucleolytic nicking⁸. However, if living cells are lysed in a detergent, 2 M salt and chelating agents, structures are released which resemble nuclei^{4,9-12}. Such nucleoids contain naked histone-free DNA packaged within a flexible cage of RNA and protein. Some

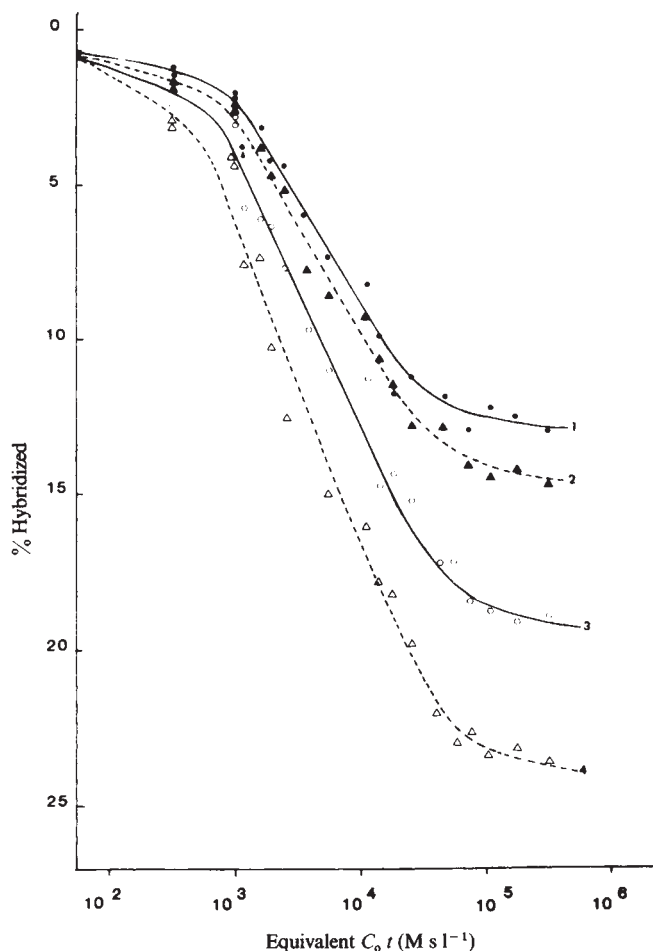


Fig. 1 Cage-associated DNA is enriched in sequences complementary to nucleoid RNA. Four different samples of cage-associated DNA containing 100% (sample 1), 35% (2), 14% (3) and 5% (4) of the DNA associated with undigested nucleoids were labelled by nick-translation, denatured and the per cent of the DNA forming a hybrid with an excess of nucleoid RNA determined. Nucleoids (prepared from cells labelled with ^3H -thymidine) were incubated with various amounts of *Eco*RI and the cages, and any associated DNA, sedimented free of unattached DNA¹⁸. The pellet was solubilized in proteinase K and SDS and the amount of DNA remaining cage associated determined by scintillation counting. The cage-associated DNA was purified, completely fragmented with *Eco*RI to yield populations of fragments with similar sizes¹⁸ and labelled by nick-translation³¹ using ^3H -dCTP (58 Ci mmol⁻¹) to yield products with specific activities of 50–70 $\times 10^6$ d.p.m. per μg . After heat denaturation, these single-stranded probes had sizes between 100 and 200 nucleotides (determined by gel electrophoresis). RNA was purified from nucleoids and hybridized in excess (RNA:DNA, 50 mg ml⁻¹:25 ng ml⁻¹) to the labelled DNA and the per cent of the DNA forming a hybrid determined essentially as described by Levitt *et al.*³². Self-annealing of the probe, in the presence of an excess of tRNA, never exceeded 2% and has been subtracted from the values presented. During the very long incubation required to achieve high equivalent C_0t values, <5% of the probe became acid soluble.

nucleoid proteins resemble those of various sub-nuclear structures isolated by other workers^{13–17}. Their DNA, which is looped by attachment to the cage, is supercoiled and so must be unbroken. Therefore, these nucleoids might be expected to contain unbroken RNA.

We first studied whether DNA close to the cage was richer than total DNA in transcribed sequences. Four types of DNA were prepared by incubating HeLa nucleoids with various amounts of *Eco*RI; then cages, and any associated DNA, were sedimented free of detached fragments to yield pellets which retained various amounts of DNA¹⁸. This cage-associated DNA was purified and hybridized with total nucleoid RNA (Fig. 1). (As nucleoids contain all the nuclear RNA, this is equivalent to nuclear RNA.) The cage-associated DNA hybridized to a

greater extent than total nuclear DNA (sample 1), for example, 23% of the DNA from cages which retained 5% of the total (that is, sample 4) is complementary to nucleoid RNA. Assuming that only one strand is transcribed, about half this sample of cage-associated DNA contains transcribed sequences—a remarkable enrichment.

We next determined whether nascent RNA was attached to the cage. When HeLa cells are incubated with ^3H -uridine for 1 or 15 min, >95% of the cellular radioactivity which is insoluble in trichloroacetic acid subsequently co-sediments with nucleoids (unpublished results and ref. 4). We tested the possibility that it is entangled in the cage network or the high concentration of DNA by determining whether it could be detached with DNA from the cages. Cells were labelled with [*Me*- ^{14}C]thymidine (0.005 $\mu\text{Ci ml}^{-1}$; 56 mCi mmol⁻¹) for 24 h, followed by ^3H -uridine (10 $\mu\text{Ci ml}^{-1}$) for 2.5 min. (Actinomycin D (0.08 $\mu\text{g ml}^{-1}$) was present during, and 30 min before, the ^3H pulse to suppress ribosomal RNA synthesis¹⁹.) Nucleoids were

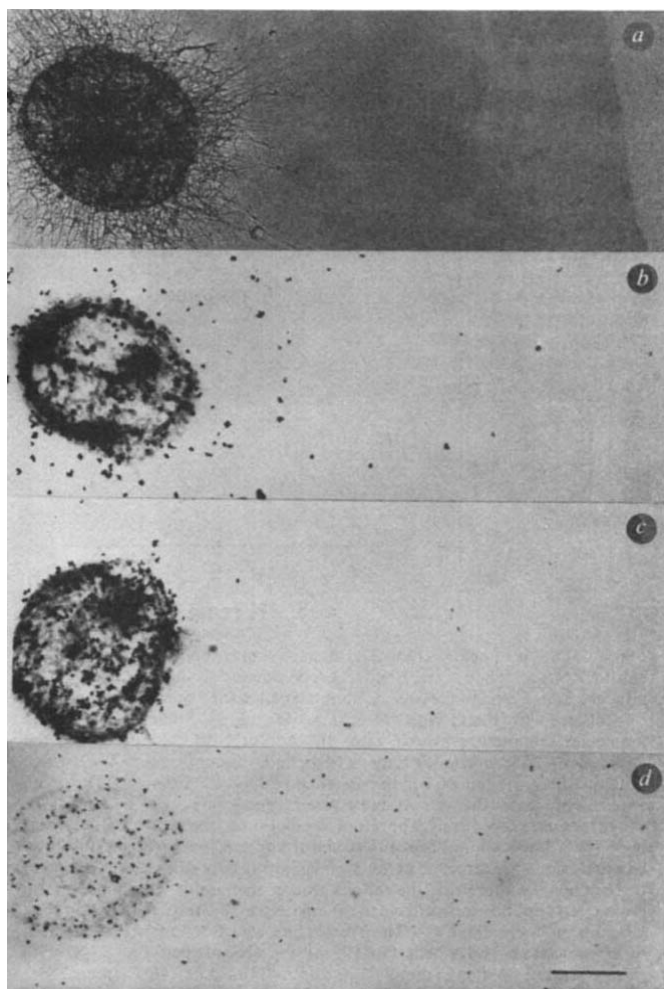


Fig. 2 RNA is attached to the cage. *a*, An electron micrograph of a nucleoid spread by Kleinschmidt's procedure¹² illustrating the cage and the skirt of tangled DNA fibres. *b–d*, Autoradiographs of spreads²⁰ after labelling: *b*, HeLa cells *in vivo* for 24 h with [*Me*- ^3H]thymidine (58 Ci mmol⁻¹; 0.05 $\mu\text{Ci ml}^{-1}$); *c*, cells *in vivo* for 2.5 min with [*5,6*- ^3H]uridine (46 Ci mmol⁻¹; 250 $\mu\text{Ci ml}^{-1}$); *d*, nucleoids *in vitro* by transcribing nucleoids for 15 min using *E. coli* RNA polymerase (see ref. 21; 3 units per assay; [*5,6*- ^3H]UTP, 52 Ci mmol⁻¹, 20 $\mu\text{Ci ml}^{-1}$). The reaction was stopped by the addition of NaCl to 2.0 M. So that silver grains can be easily seen and counted in *b–d*, DNA is shadowed but not stained and can be seen only at higher magnifications. Nascent RNA cannot have been torn from the DNA—and so lost—during the violent spreading because the same total number of grains lie over the DNA of spread and unspread nucleoids (results not shown). Scale bar, 5 μm .

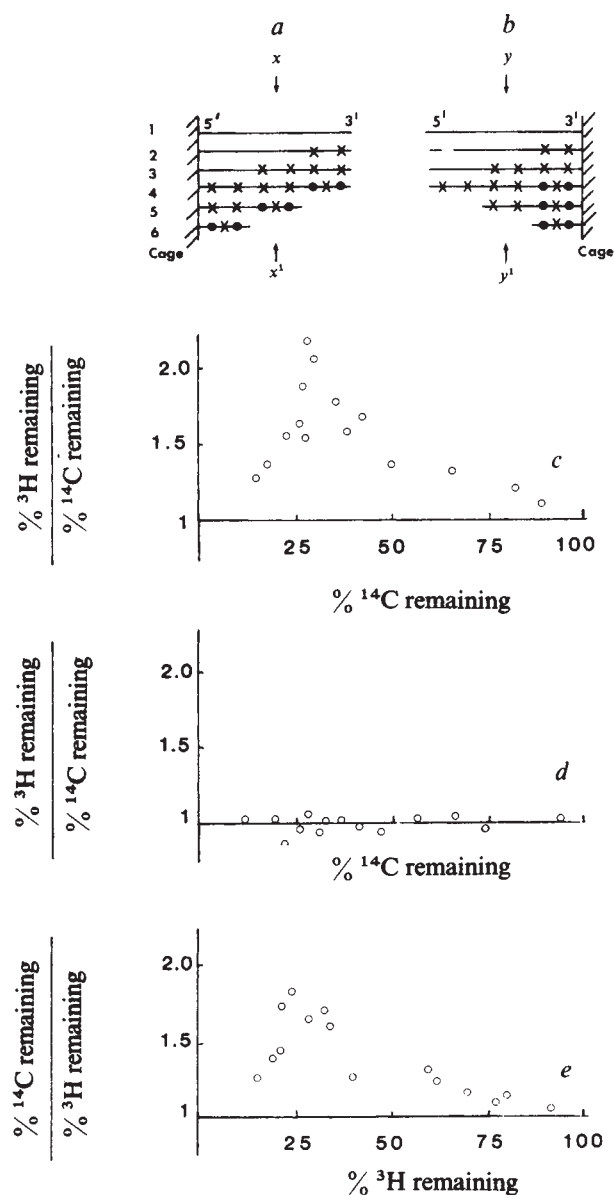


Fig. 3 RNA is attached at the 3' end. *a*, A simple model for labelling and cutting RNA attached at the 5' end. (1) A completed strand of RNA (—) is attached at its 5' end to the cage. Cells are labelled for 2 min with ¹⁴C-uridine (x) followed by 1 min with ³H-uridine (●). (2, 3), Some nascent RNA molecules complete synthesis after the addition of ¹⁴C but before the addition of ³H, so becoming only ¹⁴C-labelled, whereas others (4–6), which initiate during the pulses, become labelled with both ¹⁴C and ³H. Digestion with ribonuclease (that is, cutting between *x* and *x'*) detaches ¹⁴C and ³H in roughly equal proportions. Therefore, on digestion, the ratio (%³H remaining): (%¹⁴C remaining) remains at about unity. *b*, An array of molecules labelled as in *a* are attached at the 3' end. Cutting between *y* and *y'* detaches ¹⁴C but not ³H: therefore, the ratio is greater than unity. These models are gross oversimplifications and we use them only to illustrate whether or not a label becomes enriched. *c–e*, The 3' end of nascent RNA resists detachment by ribonuclease. HeLa cells (5×10^7 ml⁻¹) were incubated at 37°C with [U-¹⁴C]uridine (490 mCi mmol⁻¹) and [5,6-³H]uridine (46 Ci mmol⁻¹) as indicated. Actinomycin D (0.08 μg ml⁻¹) was present 30 min before, and during, the labelling. Labelling was stopped by the addition of 50 vol ice-cold phosphate-buffered saline containing 10 mM uridine, the cells were pelleted and washed, and nucleoids isolated and diluted to 0.2 M NaCl and $\sim 2 \times 10^6$ ml⁻¹ with 10 mM Tris (pH 8.0). They were then incubated with pancreatic ribonuclease (2–500 ng ml⁻¹) for 0–30 min at 37°C and the reaction was stopped by addition of NaCl to 2 M. The per cent of label remaining associated with cages was determined by filtration²⁰. Control experiments using mixtures of labelled RNA and nucleoids showed that 88% of the RNA could be filtered free of the cages. Labelling was for: *c*, 2 min with ¹⁴C-uridine (2 μCi ml⁻¹) followed by 1 min with ³H-uridine (200 μCi ml⁻¹); *d*, 3 min with both ³H-uridine (200 μCi ml⁻¹) and ¹⁴C-uridine (2 μCi ml⁻¹); *e*, 2 min with ³H-uridine (100 μCi ml⁻¹) followed by 1 min with ¹⁴C-uridine (4 μCi ml⁻¹). In these conditions sufficient label was incorporated to be counted accurately; for example, filters bearing undigested nucleoids contained about 10^4 and 2×10^3 d.p.m. of ³H and ¹⁴C, respectively.

isolated, incubated with *Eco*RI and the amount of the two labels remaining associated with cages was determined after filtering them free of detached DNA²⁰. In one typical experiment, when 90% of ¹⁴C (that is, DNA) was detached, <5% of the ³H (RNA) was lost. (A control experiment showed that >80% of the ³H but none of the ¹⁴C could be detached by ribonuclease.)

A second experiment confirms that nascent RNA is not simply entangled in DNA. When nucleoids are spread on an air/aqueous interface their DNA, initially confined within the cage, forms a huge skirt which is attached to, and surrounds, the collapsed cage¹² (Fig. 2*a*). If RNA were entangled in DNA we would expect its distribution to reflect that of spread DNA. The latter is obtained from autoradiographs of spreads prepared from cells containing uniformly labelled DNA (Fig. 2*b*): 55% of the DNA is outside the cage²⁰. However, when cells are pulse-labelled with ³H-uridine for 2.5 min, >90% of the grains lie over the cage in each of 20 spreads selected at random (Fig. 2*c*). This is so whether or not the DNA has been lightly γ ray-irradiated (9.6 J per kg) to release supercoils^{9,11} (results not shown).

The following controls demonstrate that RNA made *in vitro* can escape with the DNA from the cage and does not stick nonspecifically to the cage network. RNA was synthesized *in vitro* by incubating nucleoids with *Escherichia coli* RNA polymerase, ³H-UTP and the appropriate precursors²¹. The transcripts co-sediment with the nucleoids²¹ and are spread by Kleinschmidt's procedure; 34% of the autoradiographic grains are found over the skirt (Fig. 2*d*). Furthermore, digestion with *Eco*RI (using conditions which detached 79% of the DNA) detaches 75% of this RNA labelled *in vitro* so that it can then be filtered free of the cages. (The conditions for transcription and filtration are described in the legends to Figs 2 and 3.) This RNA made *in vitro* is not as tightly associated with the cage as that synthesized *in vivo*. We next determined whether this tight association was specific.

A 'cap' containing methylated bases is synthesized at the 5' end of nascent RNA as transcription begins^{22–24}. Therefore, ³H-methionine can be used to label caps; however, this label is also incorporated into proteins, DNA and other methylated bases within RNA chains, especially into those within ribosomal RNA molecules. We suppress the latter using actinomycin D^{19,23}. We established what proportions were incorporated into these different fractions by labelling cells for 15 min with ³H-methionine. Of the label in nucleoids, 75% was solubilized by proteinase K and SDS, and so must be in protein, and 23% was recovered in RNA (Table 1). As others have found^{25,26}, 36% of the label in RNA was in caps, the remainder being in internal residues within the original RNA chains (control sample in Table 1).

Table 1 Distribution of incorporation of ³H label into different fractions on labelling RNA

	Amount label remaining (c.p.m. $\times 10^{-3}$)	
	Control	RNase treated
Initially in nucleoids	600 (100%)	600 (100%)
In RNA	138 (23%)	68 (11%)
In internal residues in RNA	90.2 (15%)	24.6 (4.1%)
In caps in RNA	50.0 (8.3%)	48.2 (8.0%)

Cells were labelled for 15 min with L-[Me-³H]methionine (50 μCi ml⁻¹; 78 Ci mmol⁻¹), and nucleoids isolated and diluted to 0.2 M NaCl and 2×10^6 ml⁻¹ with 10 mM Tris (pH 8.0)^{4,11}. Actinomycin D (0.08 μg ml⁻¹) was present 30 min before, and during, the labelling. Nucleoids were then incubated with or without pancreatic ribonuclease (5 μg ml⁻¹) for 15 min at 37°C, the reaction was stopped by the addition of NaCl to 2 M and the nucleoids were pelleted through a cushion of 7.5% sucrose containing 2 M NaCl. The pellet was digested with deoxyribonuclease (5 μg ml⁻¹) for 20 h and then proteinase K and SDS¹⁸. Next, RNA was purified³⁰ and the proportion of label in caps and internal residues in the RNA was determined after digestion with RNase A and T2 followed by column chromatography: mononucleotides and caps eluted in fractions containing +1 and +5 charges, respectively^{23,25,26}. The amount of ³H in the different fractions is given in the table with percentages in parentheses. The amount of RNA detached (75%) by the ribonuclease from the pelleted cages was determined from an identical experiment in which cells were labelled with [5,6-³H]uridine (46 Ci mmol⁻¹; 10 μCi ml⁻¹) rather than the ³H-methionine.

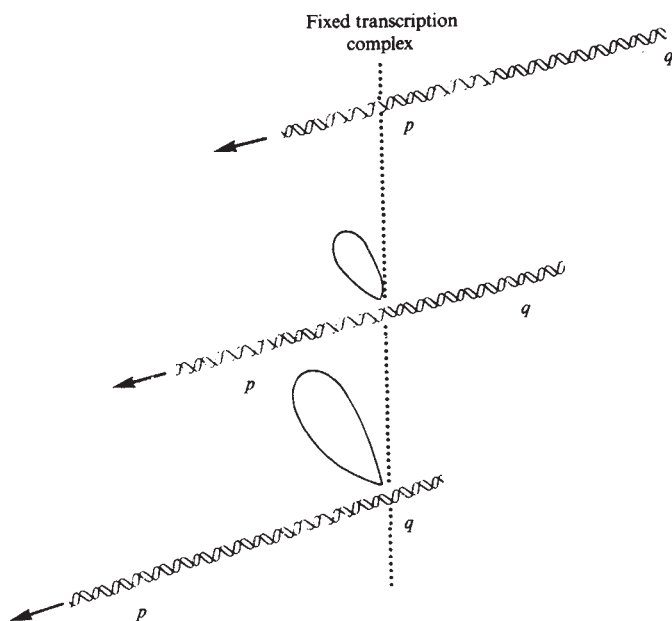


Fig. 4 A model for transcription. *a*, A fixed complex transcribes DNA between *p* and *q*. *b, c*, RNA is synthesized as DNA passes through the complex, the nascent RNA chain being attached at both ends forming a loop. Transcription of a helical template—itsself attached to the cage—to yield attached transcripts poses a number of topological problems. If the polymerase rotates about the helical axis then the transcript can be separated from its template during synthesis by cutting one and passing the other through the cut (there is a precedent for cutting RNA during 'splicing'). Alternatively, a fixed topological relationship might be maintained between the cap and 3' end, perhaps by attaching both to the polymerase, so that if one rotates then so does the other. If DNA rotates, then supercoiling is inevitably induced elsewhere in the DNA and must be stored or released (for example by a nicking-closing activity).

If nascent RNA is attached at its 5' end, caps should resist detachment by ribonuclease. Therefore, we incubated ^3H -methionine-labelled nucleoids with sufficient ribonuclease to detach 75% of nascent RNA. Next, cages and any associated RNA were sedimented free of detached RNA and the amounts of label in the different fractions determined (Table 1). The ribonuclease treatment did not affect the quantity of label recovered in protein or DNA (results not shown) whereas it halved that in RNA. This reduction was almost entirely due to the loss of label in internal residues with negligible loss in caps. Therefore, removal of 75% of the body of the chain detaches few, if any, caps.

We have attempted to demonstrate attachment at the 3' end as follows. Cells were incubated for 2 min with ^{14}C -uridine and then for 1 min with ^3H -uridine. The doubly labelled nucleoids were then incubated with ribonuclease, the detached RNA removed by filtration and the percentage of the labels remaining associated with the cages determined. If RNA is attached at random, ^{14}C and ^3H will be detached from the cages in equal proportions, that is, the normalized ratio (% ^3H remaining): (% ^{14}C remaining) will remain at unity independently of the amount of ^{14}C remaining. On the other hand, if the 3' end is attached, detachment of RNA will lead to a relative enrichment of ^3H : the ratio will increase above unity as the amount of ^{14}C remaining decreases (Fig. 3*a, b*). (Attachment at both ends cannot be distinguished from 3' attachment using this labelling regime.) The results are consistent with attachment at the 3' end because the ^3H resists detachment. For example, removal of all but 30% of ^{14}C leaves 54% of ^3H (thus, the ratio in Fig. 2*c* is 54:30 = 1.8). Experiments with simultaneous labelling and label reversal (Fig. 3*d, e*) rule out labelling artefacts.

Three points should be borne in mind in discussing these results. First, we are concerned mainly with heterogeneous

nuclear RNA as we have generally labelled in actinomycin D to suppress ribosomal RNA synthesis. Second, our pulse times should label many unfinished molecules. [Heterogeneous nuclear RNA—1,500–50,000 nucleotides²³—is synthesized at ~5,000 nucleotides per min (ref. 27)]. The third point concerns the anchorage by polymerases of 3' ends to DNA and so to the cage. We would expect polymerases to dissociate from the DNA in the 2 M NaCl used to make nucleoids and although nucleoids contain no polymerase activity²¹, we cannot preclude anchorage by some—perhaps undiscovered—subunit of the polymerase. However, if polymerase does hold the 3' ends in this way, it must do so close to the cage because neither transcript nor its template is readily detached by *EcoRI*.

We have demonstrated that transcribed sequences are closely associated with a sub-nuclear structure that we call the nuclear cage. Furthermore, nascent RNA is attached at the 5' cap—and perhaps at the 3' end—to the cage. The striking micrographs illustrating polymerases processing along the DNA unattached to any larger structure are obtained only after disruptive spreading or with unusually hyperactive genes which may be very special cases. (For example, lampbrush loops and amplified ribosomal genes in amphibian oocytes.)

A trivial explanation is consistent with our results. Perhaps nascent RNA, which is synthesized throughout the nucleus *in vivo*, sticks to the cage when nucleoids are prepared. We think this extremely unlikely: nearly all this RNA would have to stick specifically at the 5' end and remain stuck during filtration or spreading, even though the control experiments show that RNA synthesized *in vitro* has little if any, affinity for nucleoids. A second explanation is that a polymerase initiates at the cage and moves away to yield a transcript attached at its cap. However, this cannot readily explain why, in the presumed absence of polymerase, the 3' end resists detachment by ribonuclease. Alternatively, transcripts may be generated as DNA passes through a fixed transcription complex to yield transcripts attached at both ends (Fig. 4). Whichever proves to be correct, it seems that the cage must have an important role in transcription. As replication is initiated by attaching sequences to the cage²⁰ (see also refs 28, 29), perhaps genes are also activated during differentiation by attachment.

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1. Miller, O. L. & Beatty, B. R. *Science* **164**, 955–957 (1969).
2. McKnight, S. & Miller, O. L. *Cell* **8**, 305–319 (1976).
3. Franke, W. W. *Int. Rev. Cytol. Suppl.* **4**, 71–236 (1974).
4. Cook, P. R., Brazell, I. A. & Jost, E. J. *Cell Sci.* **22**, 303–324 (1976).
5. Miller, T. E., Huang, C.-Y. & Pogo, A. O. J. *Cell Biol.* **76**, 675–691 (1976).
6. Herman, R., Weymouth, L. & Penman, S. J. *Cell Biol.* **78**, 663–674 (1978).
7. Harlan, G., Echert, W. A., Kaffenberger, W. & Wunderlich, F. *Biochemistry* **18**, 1782–1788 (1979).
8. Warren, A. C. thesis, Univ. Oxford (1977).
9. Cook, P. R. & Brazell, I. A. J. *Cell Sci.* **19**, 261–279 (1975).
10. Cook, P. R. & Brazell, I. A. J. *Cell Sci.* **22**, 287–302 (1976).
11. Cook, P. R. & Brazell, I. A. *Eur. J. Biochem.* **84**, 465–477 (1978).
12. McCready, S. J., Akkrig, A. & Cook, P. R. J. *Cell Sci.* **39**, 53–62 (1979).
13. Berezney, R. & Coffey, D. S. *Biochem. biophys. Res. Commun.* **60**, 1410–1417 (1974).
14. Riley, D. E., Keller, J. M. & Byers, B. *Biochemistry* **14**, 3005–3013 (1975).
15. Aronson, R. P. & Blobell, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1007–1011 (1975).
16. Comings, D. E. & Okada, T. A. *Exptl Cell Res.* **103**, 341–360 (1976).
17. Levin, J. M. thesis, Univ. Oxford (1978).
18. Cook, P. R. & Brazell, I. A. *Nucleic Acids Res.* **8**, 2895–2906 (1980).
19. Perry, R. P. *Exptl Cell Res.* **29**, 400–406 (1963).
20. McCready, S. J., Godwin, J., Mason, D. W., Brazell, I. A. & Cook, P. R. J. *Cell Sci.* **46**, 365–386 (1980).
21. Colman, A. C. & Cook, P. R. *Eur. J. Biochem.* **76**, 63–78 (1977).
22. Furuchi, Y. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1086–1090 (1978).
23. Salditt-Georgieff, M., Harpold, M., Chen-Kiang, S. & Darnell, J. E. *Cell* **19**, 68–78 (1980).
24. Babich, A., Nevins, J. R. & Darnell, J. E. *Nature* **287**, 246–248 (1980).
25. Perry, R. P., Kelley, D. E., Friderici, K. & Rottman, F. *Cell* **4**, 387–394 (1975).
26. Wei, C.-M., Gershowitz, A. & Moss, B. *Cell* **4**, 379–386 (1975).
27. Cox, R. F. *Cell* **7**, 455–465 (1976).
28. Dijkwel, P. A., Mullenders, L. H. F. & Wanka, F. *Nucleic Acids Res.* **6**, 219–230 (1979).
29. Pardoll, D. M., Vogelstein, B. & Coffey, D. S. *Cell* **19**, 527–536 (1980).
30. Palmiter, R. D. *Biochemistry* **13**, 3606–3615 (1974).
31. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184–1188 (1975).
32. Levitt, A., Axel, R. & Cedar, H. *Dev. Biol.* **69**, 496–505 (1979).

Developmental expression of a *Drosophila* actin gene encoding actin I

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The *Drosophila melanogaster* actin genes comprise a dispersed multigene family^{1,2} of six genes, one at each of six scattered chromosomal loci (refs 1, 2 and unpublished observations). These genes encode the *Drosophila* actins I, II and III^{3,4}, which can be separated in the isoelectric focusing dimension of two-dimensional gels. Actins II and III have been found in all tissues, with actin III an unstable species except in adult thorax⁵, while actin I, the most acidic form, appears during larval myogenesis both *in vivo* and *in vitro*^{3,4}. Some actins therefore seem to be developmentally regulated. If individual members of a multigene family are regulated at the transcriptional level, this implies that developmental mechanisms of gene regulation can be elucidated by comparing gene family members. We report here a pattern of transcriptional activity of the *D. melanogaster* actin gene at the 79B chromosomal locus. Our results suggest that the 79B actin gene encodes actin I, the larval muscle-specific actin, and that actin I is synthesized initially as a precursor. The precursor co-migrates with cytoplasmic actin at the position of actin II and is subsequently acetylated to form actin I.

Because actin is a highly conserved protein⁶, the DNA sequences encoding the protein cross-react with great efficiency, both within actin gene family members of a particular organism and between different species^{1,7-10}, and so the gene sequences themselves cannot be used to detect the transcript of any one individual gene. We therefore isolated a specific probe from the transcribed, but non-translated 3' portion of the 79B actin gene and used it to investigate the transcriptional activity of the 79B gene at different stages of *Drosophila* development. We also used this probe to select homologous mRNA from a heterogeneous mRNA population. The message which bound to this

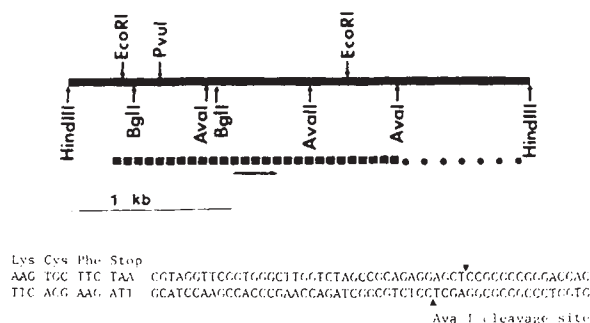


Fig. 1 Physical map of the actin gene at the 79B cytological locus. This 2.8-kb *HindIII* DNA fragment from phage λ insert DNA¹¹ containing the 79B actin gene was subcloned into the plasmid pBR322 as described previously¹. The locations of restriction endonuclease sites within the plasmid insert are indicated. The dashed line and arrow indicate the amino acid-encoding region of the plasmid and direction of transcription as determined by DNA sequence analysis¹⁴. The *AvaI*-*HindIII* fragment at the 3' end of the gene delineated by the dotted line was used as the gene-specific probe for the 79B actin gene. The DNA sequence from the 3' end of the amino acid-coding region to the *AvaI* restriction endonuclease cleavage site at the beginning of the gene-specific probe is shown.

probe was then eluted, translated *in vitro* and the translation products electrophoresed on two-dimensional gels to distinguish between actins I, II and III.

Several clones representing the 79B actin gene were isolated from a bank of Canton S DNA cloned into phage λ by Maniatis and co-workers¹¹, using our original actin clone *Kla* (ref. 1) as probe. The presumptive coding region of each as identified by homology to *Kla* was subcloned into pBR322. The 79B cytological locus of origin¹² was established by *in situ* hybridization¹³ of a ³H-labelled cRNA probe synthesized using as template a subclone representing a region flanking the actin protein-coding

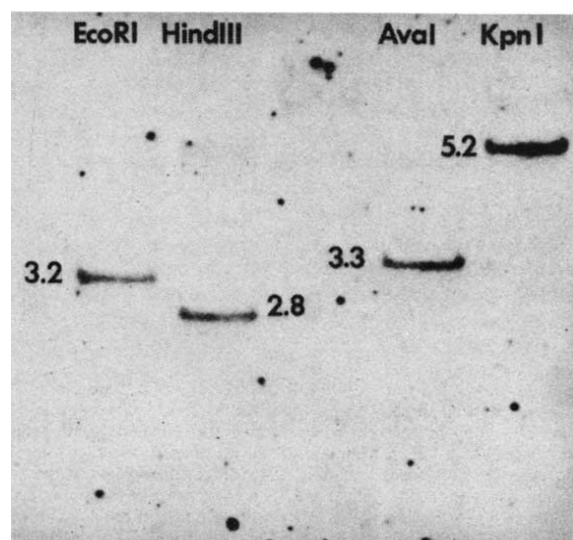


Fig. 2 Autoradiogram illustrating hybridization of the 79B gene-specific probe to genomic DNA from Canton S embryos. The 79B gene-specific probe was isolated from a preparative digest of whole plasmid DNA¹, labelled with ³²P by nick-translation¹⁵ and hybridized to Canton S embryo DNA that had been cut with the indicated restriction endonucleases, electrophoresed in a 1% agarose gel and transferred to a nitrocellulose filter²⁵. The sizes of the labelled fragments (kb) are indicated (exposure 4 days).

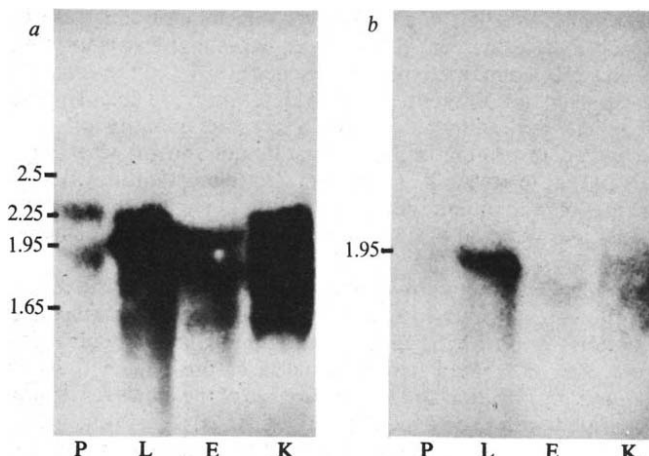


Fig. 3 Autoradiogram illustrating hybridization of coding region (a) and gene-specific (b) probes to poly(A) RNAs from embryos, larvae, pupae and Kc cells. Probes were labelled by nick-translation¹⁵ and hybridized to poly(A) RNAs¹ that had been denatured, separated on a single methyl mercury-containing agarose gel²⁶ and covalently bound to a diazobenzoyloxymethyl paper filter¹⁷. a, Hybridization of coding region probe to filter. This probe was the 1.8-kb *HindIII* fragment from the 5C actin gene which contains the complete actin amino acid-coding region of that gene. This gene was one of those we have isolated from the Maniatis Canton S genome bank¹¹ and is homologous to that previously described². The probe was removed by several washes with 95% formamide at 45 °C. b, Hybridization of 79B gene-specific probe to filter. The sizes of the labelled transcripts (kb) are indicated. Embryos (E); larvae (L); pupae (P); Kc cells (K).

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sequence¹. All 79B clones were characterized by an *Eco*RI restriction endonuclease cleavage site within an intervening sequence starting at amino acid 307 (data to be presented elsewhere).

Figure 1 shows a restriction endonuclease cleavage map of the 79B actin gene. The gene-specific probe is indicated and the *Ava*I restriction endonuclease site at which it begins has been localized by DNA sequence analysis¹⁴ at 30 nucleotides beyond the 3' end of the amino acid coding region. We have used the fragment indicated in Fig. 1 for the experiments described here. This DNA fragment contains no amino acid-encoding nucleotides and, as shown in Fig. 2, hybridizes to only one genome DNA fragment with a molecular weight corresponding to that of the original clone fragment from which it was derived. This may be contrasted with the seven fragments labelled when a similar blot of *Eco*RI-digested Canton S genome DNA is hybridized to an amino acid-coding region probe².

The gene-specific probe was labelled with ³²P by nick-translation¹⁵ and hybridized to populations of poly(A)RNAs isolated from three different stages of *Drosophila* development and from Kc cells¹⁶ which had been fractionated on an agarose gel and transferred to diazotized paper¹⁷. Figure 3 contrasts the reaction of a coding region probe which will hybridize to all actin transcripts with the hybridization of the 79B gene-specific probe. Actin transcripts of varying lengths (1.65–2.5 kilobases (kb)) can be observed in the preparations while the 79B gene-specific probe hybridizes strongly to only one RNA species (1.95 kb) from early third instar larvae and weakly to RNA from Kc cells. Because the transcripts homologous to the 79B gene-specific probe constitute a significant proportion only of larval actin message, the 79B gene seems to function independently of at least some other actin genes. This experiment establishes developmental specificity of the transcriptional activity of the 79B locus.

To determine whether actins I, II or III are synthesized by the 79B actin gene, we immobilized the gene-specific probe DNA on nitrocellulose, hybridized the probe DNA to homologous poly(A) RNA from early third instar larvae, eluted the bound RNA¹⁸ and translated the RNA *in vitro*¹⁹. Figure 4a, b shows the translation products after separation by two-dimensional electrophoresis²⁰. Before selection of the 79B gene-specific transcripts, actins I, II and III are observed (Fig. 4a). In contrast, translation products of the RNA homologous to the 79B gene-specific probe migrate at the positions of actins I and II (Fig. 4b). These results differ from those obtained with translation

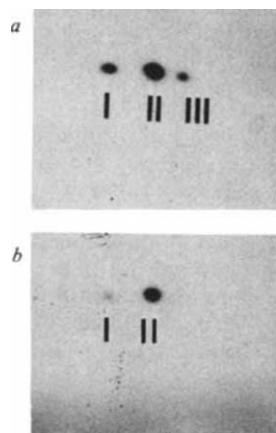


Fig. 4 Autoradiograms of two-dimensional gel electrophoresis of ³⁵S-methionine-labelled translation products of early third instar larval RNAs. *a*, Poly(A) RNA¹ from early third instar larvae was translated *in vitro*¹⁹ and the translation products electrophoresed in two dimensions²⁰. *b*, The same RNA was hybridized to DNA of the 79B gene-specific probe and the bound RNA eluted¹⁸. This RNA was then translated and electrophoresed simultaneously with shared buffer chambers with gel *a*. Actins I, II and III are indicated.

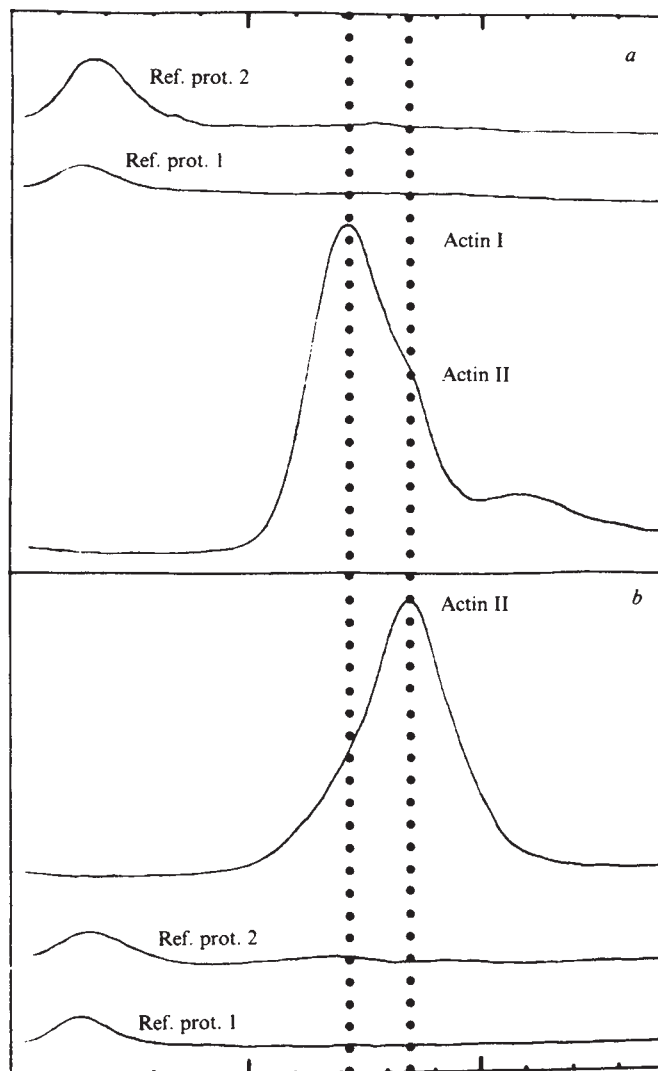


Fig. 5 Densitometer scan of an autoradiogram of two-dimensional gel electrophoresis of ³⁵S-methionine-labelled translation products with and without reagents which prevent acetylation of the *in vitro* synthesized proteins²⁴. Total RNA was isolated from early third instar larvae¹ and translated *in vitro*¹⁹. The translation products were electrophoresed simultaneously with shared buffer chambers in two dimensions²⁰ and autoradiographed. The X-ray films were scanned with a soft laser scanning densitometer (Biomed Instruments). Two reference proteins from each gel are included. *a*, Densitometer scan of actins and two reference proteins which were translation products of total RNA from early third instar larvae. *b*, Densitometer scan of actins and two reference proteins which were translated from the same RNA preparation in the presence of citrate synthase (0.88 units per 25-μl reaction solution) and oxaloacetate (1 mM) as described by Palmer²⁴.

products (actins II and III) of RNAs selected by a cytoplasmic *Drosophila* actin gene probe². Thus, either the gene-specific probe selects more than one message or the two actins observed are related by a precursor-product relationship. We think the first hypothesis unlikely because the probe does not hybridize to other actin genes (see Fig. 2).

If the isoelectric point shift between actin II and actin I were due to post-translational processing, acetylation would seem a likely candidate for several reasons. Actin III is converted into actin II by an acetylation²¹ in Kc cells, causing a shift in isoelectric point from 5.84 to 5.77 (ref. 3). As the isoelectric points of actin I (5.70) and actin II (5.77) also differ by 0.07 pH units, acetylation of actin II could account for its conversion into actin I. The reticulocyte lysate has a ribosome-associated trans-acetylase²² which is capable of acetylating *Dictyostelium* actin²³.

Also, a proportion of the *in vitro* acetylation of proteins in the reticulocyte translation system can be suppressed by the addition of oxaloacetate and citrate synthase²⁴. Consequently, we carried out *in vitro* translation of total larval RNA with and without oxaloacetate and citrate synthase and separated the products by two-dimensional electrophoresis. The results of this experiment are shown in Fig. 5a, b. When acetylation is suppressed to this degree, actin I is greatly reduced, presumably because of diminished conversion of actin II into actin I. The peak of actin II is increased, presumably due to accumulation of actin II which would ordinarily have been acetylated into actin I. These results strongly suggest that actin I is synthesized as a component that initially co-migrates with acetylated cytoplasmic actin and is subsequently acetylated. The acetylation of a precursor can thus account for the characteristic isoelectric point and position on two-dimensional gels of actin I^{3,4}, and can also explain why the turnover of actin III in muscle cells is insufficient to account for the quantity of actin II observed^{3,4}. In addition, this result suggests the evolutionary conservation of mechanisms of acetylation because the rabbit acetylase is apparently able to recognize and acetylate *Drosophila* actins.

Thus, we believe the 79B actin gene encodes actin I, the larval muscle-specific actin, although it does not seem to be the only actin gene to do so (unpublished data). Several characteristics of the transcript of this gene are consistent with this hypothesis; for example, the stage at which the gene is active, the difference in size of the transcript from that of one of the cytoplasmic messages² and the synthesis of actin I by *in vitro* translation of mRNA homologous to the 79B gene-specific probe.

The 79B locus seems to be developmentally regulated independently of other *Drosophila* actin genes (Fig. 3). Differential control of actin gene expression during development may at least partly explain the multiplicity of actin genes which have been found in several eukaryotes^{7,8}. We are continuing to develop gene-specific probes for the remainder of the actin genes of *D. melanogaster* to investigate the pattern(s) of gene expression and form of actin synthesized by each. These patterns of expression will ultimately be correlated with DNA sequence information from the 5' regions of each actin gene so as to define the sequences responsible for this regulation.

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Gene required in G₁ for commitment to cell cycle and in G₂ for control of mitosis in fission yeast

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The control regulating commitment to the cell division cycle of eukaryotes seems to occur before the initiation of DNA replication¹⁻⁴. In the budding yeast *Saccharomyces cerevisiae*, this control is called start and is the earliest gene-controlled event of the cell cycle³⁻⁵. A haploid cell which has completed start is committed to cell division and unable to undergo alternative developmental fates such as conjugation. Here, we describe an analogous start control in the fission yeast *Schizosaccharomyces pombe*. We have tested the ability of *cdc* mutants blocked at various stages of the cell cycle to undergo conjugation. Only mutants of *cdc 2* and *cdc 10* which block during the G₁ period are able to conjugate. Other mutants which block during G₁, S phase, G₂ or mitosis are committed to cell division and cannot conjugate. The commitment control start is located in G₁, and its completion requires the gene functions of *cdc 2* and 10. Completion of start occurs at the beginning of the cell cycle in rapidly growing cells, but is delayed to later in the cell cycle at slow growth rates. The *cdc 2* gene function also participates in another major cell cycle control which determines the timing of mitosis⁶⁻⁸. Therefore *cdc 2* is a cell cycle control gene which acts at two separate points in the cell cycle: it is required in G₁ for commitment to cell division and in G₂ for the control of mitosis.

When dividing *S. pombe* cells are deprived of nitrogen, they stop proliferating, accumulate in G₁ and, if they are of appropriate mating type, undergo conjugation and sporulation^{9,10}. To determine whether conjugation is limited to a specific phase of the *S. pombe* cell cycle, various temperature-sensitive *cdc* mutants were incubated at their restrictive temperature to block cells at different places in the cell cycle, and the ability of these cells to conjugate was tested. The restrictive temperature of the *cdc* mutants was either 35 or 36 °C (refs 11, 12, 20), but at these temperatures conjugation in wild type was inhibited. As the highest temperature at which a reasonable level of conjugation took place was 33 °C (ref. 10), the experiment was carried out by incubating the *cdc* mutant cells for two generations at 36 °C to block progress through the cell cycle, followed by shifting the cells to 33 °C and mixing them with cells of opposite mating type to allow conjugation to take place. After overnight incubation the mating mix was plated out to assay the number of diploid cells formed as a consequence of conjugation (for practical details see Table 1 legend). In these conditions of temperature and medium, mutants of 11 of the 16 *cdc* genes known to be required for DNA replication or mitosis were blocked in their progress through the cell cycle. Mutants of the other five genes were not completely blocked and could not be used in the experiment. The levels of conjugation expressed as a percentage of a wild-type control are given in Table 1 (column 1). Only mutants of *cdc 2* and 10 gave high levels of conjugation. Other mutants were <5% of the wild-type control.

Before concluding that all the *cdc* genes apart from *cdc 2* and 10 block at cell cycle stages where conjugation is not possible, it is necessary to eliminate various other explanations for the failure of conjugation in the blocked mutants. The first possibility is that the viability of the *cdc* mutants is dramatically reduced after incubation at the restrictive temperature. This was not the case because the viabilities of the *cdc* mutants after 5 h at

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1. Tobin, S. L., Zulauf, E., Sanchez, F., Craig, E. A. & McCarthy, B. J. *Cell* **19**, 121-131 (1980).
2. Fyrberg, E. A., Kindle, K. L., Davidson, N. & Sodja, A. *Cell* **19**, 365-378 (1980).
3. Storti, R. V., Horovitch, S. J., Scott, M. P., Rich, A. & Pardue, M. L. *Cell* **13**, 589-598 (1978).
4. Fyrberg, E. A. & Donady, J. J. *Dev Biol.* **68**, 487-502 (1979).
5. Horovitch, S. J., Storti, R. V., Rich, A. & Pardue, M. L. *J. Cell Biol.* **82**, 86-92 (1979).
6. Pollard, T. D. & Weihing, R. R. *CRC Crit. Rev. Biochem.* **2**, 1-65 (1974).
7. Kindle, K. L. & Firtel, R. A. *Cell* **15**, 763-778 (1978).
8. Cleveland, D. W. *et al. Cell* **20**, 95-106 (1980).
9. Gallwitz, D. & Sures, I. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2546-2550 (1980).
10. Ng, E. & Abelson, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3912-3916 (1980).
11. Maniatis, T. *et al. Cell* **15**, 687-701 (1978).
12. Bridges, C. B. *J. Hered.* **26**, 60-64 (1935).
13. Bonner, J. J. & Pardue, M. L. *Chromosoma (Berl.)* **58**, 87-99 (1976).
14. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
15. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
16. Craig, E. A., McCarthy, B. J. & Wadsworth, S. C. *Cell* **16**, 575-588 (1979).
17. Alwine, J. C., Kemp, D. J. & Stark, C. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350-5354 (1977).
18. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4927-4931 (1979).
19. Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247-256 (1976).
20. O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).
21. Berger, E. *et al. Biochem. Genet.* **19**, 321-331 (1981).
22. Traugh, J. A. & Sharp, S. B. *J. biol. Chem.* **252**, 3738-3744 (1977).
23. Rubenstein, P. & Deuchler, J. *J. biol. Chem.* **254**, 11142-11147 (1979).
24. Palmiter, R. D. *J. biol. Chem.* **252**, 8781-8783 (1977).
25. Southern, E. M. *J. molec. Biol.* **98**, 503-518 (1975).
26. Bailey, J. M. & Davidson, N. *Analyt. Biochem.* **72**, 413-427 (1976).

36 °C and overnight at 33 °C were high, the lowest values being ~20% for *cdc* 24.M38 and *cdc* 6.23 (column 4 of Table 1). A second possibility is that the *cdc* mutants are partially sterile and have a reduced ability to mate even at the permissive temperature. This was not the case either, because at 25 °C all the mutants mated well, the lowest being ~30% of the wild-type control for *cdc* 17.K42 and *cdc* 25.22 (column 2 of Table 1). A third, more subtle, possibility is that the *cdc* mutant function is directly required in the conjugation process, and that this is the reason conjugation is prevented at the restrictive temperature (for a fuller discussion of this point see ref. 5). This possibility was tested by first accumulating the *cdc* mutant cells at the appropriate cell cycle stage for mating by incubation at 25 °C in nitrogen-free medium, shifting the cells to 33 °C and then mixing them with cells of opposite mating type. In these conditions, all the *cdc* mutants underwent conjugation, demonstrating that they were not directly involved in the conjugation process (column 3 of Table 1). Two mutants, *cdc* 1.7 and *cdc* 25.22, did have rather reduced conjugation frequencies, ~10–15%, but these levels were still far greater than their conjugation frequencies at 36/33 °C.

These experiments demonstrate that mutants of *cdc* 17, 22, 23, 24 (involved in G₁ or S phase) and of *cdc* 1, 6, 13, 25, 27 (involved in G₂ or mitosis) block at stages in the cell cycle where conjugation is not possible whereas mutants of *cdc* 2 and 10 block at a stage where it is possible. Two different mutant alleles of *cdc* 2 and 10 behave similarly (Table 1), indicating that the ability to conjugate at the restrictive temperature was not due to the presence of a second mutation in the strains. The stage at which *cdc* 10 mutant cells become blocked is during G₁, before the initiation of S phase^{11,12}. The situation with *cdc* 2 is more complex as *cdc* 2 mutants were previously thought to become blocked only in late G₂ just before mitosis^{8,11}. However, *cdc* 2

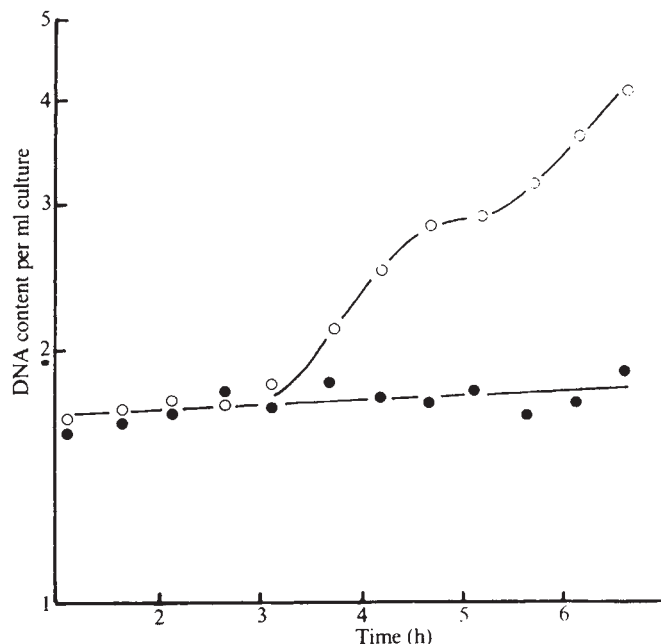


Fig. 1 *cdc* 2.33 h⁻ and 972 h⁻ wild-type cells were incubated in minimal medium lacking a nitrogen source at 25 °C and were re-inoculated into fresh minimal medium at 36 °C at time = 0 h. The DNA contents in the two cultures were estimated using the diphenylamine reaction⁶. Both cultures began with cells containing the G₁ content of DNA. DNA contents per ml are plotted on an arbitrary log scale, and the actual DNA content per ml equivalent to 1 unit on the scale is given in parentheses following the symbol key. ○, 972 wild type (76 ng); ●, *cdc* 2.33 (68 ng).

Table 1 % Conjugation for each temperature regime

<i>cdc</i> mutant	36/33 °C	25/25 °C	25/33 °C	% Viability	Can cells conjugate at this block?
<i>cdc</i> 1.7	0.046	76.2	10.4	47.6	No
<i>cdc</i> 2.33	30.9	106	105	97.1	
<i>cdc</i> 2.M63	19.0	112	129	32.7	
<i>cdc</i> 6.23	3.09	52.6	37.4	20.8	No
<i>cdc</i> 10.129	138	94.6	88.2	78.1	
<i>cdc</i> 10.K28	108	101	117	56.4	
<i>cdc</i> 13.117	0.205	60.3	33.5	59.4	No
<i>cdc</i> 17.K42	0.295	29.0	31.9	58.7	
<i>cdc</i> 22.M45	4.44	51.2	42.8	87.3	
<i>cdc</i> 23.M36	2.93	72.0	51.0	65.8	No
<i>cdc</i> 24.M38	2.83	80.3	119	19.2	
<i>cdc</i> 25.22	0.086	36.7	14.0	57.6	
<i>cdc</i> 27.K8	4.98	93.9	78.6	56.9	No

The *cdc* h⁻ mutant cultures were grown at 25 °C in minimal medium⁶⁻⁸, washed and resuspended in minimal medium lacking the nitrogen source, and incubated at 25 or 36 °C. After 5 h cells were mixed with a ×10 excess of *ura* 1 *mei* 1-B102 cells¹⁰, the 25 °C culture was split and incubated at 25 °C and 33 °C, and the 36 °C culture incubated at 33 °C. After overnight incubation the mating mix was sonicated and plated onto minimal medium at 36 °C. Only complemented diploids derived from a conjugation event between the *cdc* h⁻ and *ura* 1 *mei* 1-B102 parents could form colonies in these conditions. All the *cdc* mutants were recessive to wild type. The mating type allele *mei* 1-B102 (also known as *mat* 2-Pm) was used as this allows conjugation with cells of h⁻ mating type but prevents meiosis and sporulation. Consequently a stable diploid is formed. In each batch of experiments a control *ade* 6 h⁻ culture was also crossed to *ura* 1 *mei* 1-B102 to determine the numbers of diploids formed from wild-type cells which were not blocked in cell cycle progress by a *cdc* mutation. The levels of conjugation quoted here are expressed as percentages of this wild-type control under the temperature regime used. The per cent viabilities of the *cdc* mutants were calculated from comparison of particle and colony counts after cultures had been incubated in minimal medium lacking the nitrogen source for 5 h at 36 °C followed by overnight incubation at 33 °C.

mutant cells actually become blocked in G₁ as well as G₂, as is shown by the following experiment. *cdc* 2.33 cells were accumulated in G₁ by nitrogen starvation at 25 °C and a synchronous culture prepared by re-inoculating the cells into fresh medium at 36 °C. In these conditions wild-type cells undergo a synchronous round of DNA replication, but this does not occur in *cdc* 2.33 (Fig. 1). Thus, *cdc* is required independently for the two major events of the cell cycle, S phase and mitosis.

If *cdc* 2 has two cell cycle block points, it is likely that only those cells blocked at the first point in G₁ are able to undergo conjugation. If this was the case it would account for the reduced conjugation of *cdc* 2 mutants compared with *cdc* 10 (Table 1, column 1), as the long G₂ period found in *S. pombe* would result in most cells accumulating at the second block point in late G₂ where they could not conjugate. To test this, a synchronous culture of *cdc* 2.33 was prepared by selection of cells early in the cell cycle (mostly at the beginning of G₂), using sedimentation rate centrifugation. This culture was grown at 25 °C, and aliquots were shifted at various times during the cell cycle to 36/33 °C following the procedure described in the earlier experiments, to measure the ability of the cells to conjugate. If there is only one block point in the cell cycle, regardless of when the shift up occurred, the level of conjugation should have been the same. However, if there are two block points, one at which cells could conjugate and one at which they could not, there should have been a peak in the level of conjugation at the former block point. This second pattern was the one observed, with the mid-point of the peak occurring at the very beginning of the cell cycle coincident with cell division (Fig. 2). This is close to the timing of the G₁ period, indicating that conjugation is possible only at the G₁ block point.

We conclude that there is a start control in fission yeast which functions during the G₁ period. Before start, cells can still conjugate but after start they become committed to cell division and are unable to conjugate. Completion of start and commitment to the cell cycle requires the genes *cdc* 2 and 10. Rapidly growing cells complete the *cdc* 10 gene function very early, immediately after mitosis, but in more slowly growing cells the

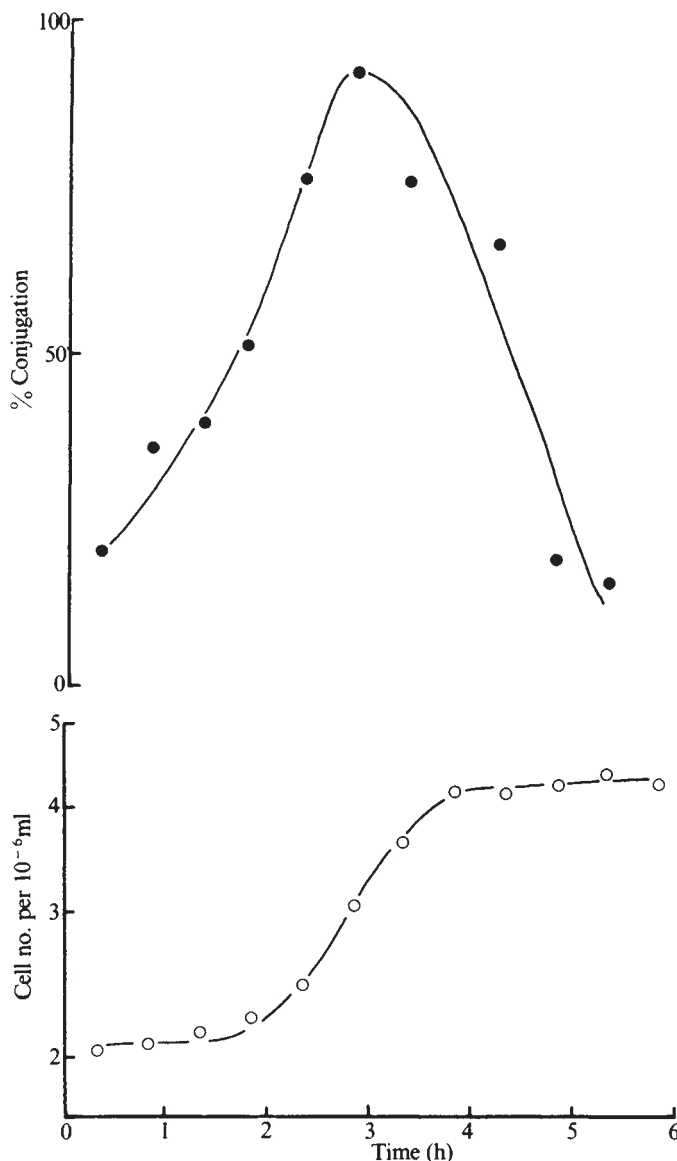


Fig. 2 A synchronous culture of *cdc 2.33 h⁻* was prepared from an exponentially growing culture by sedimentation rate velocity centrifugation of the cells through a 7.5–30% lactose gradient to isolate the small cells at the beginning of the cell cycle⁶. The synchronous culture was grown in minimal medium at 25 °C and aliquots were shifted at intervals to 36 °C for 5 h in minimal medium lacking a nitrogen source, before shifting to 33 °C and mixing with *ura 1 mei 1-B102*. The mixing and subsequent processing were as described in Table 1 legend. Cell number was measured using a Coulter counter⁶. ●, % conjugation; ○, cell number per ml.

cdc 10 function is completed much later in the cell cycle^{11–13}. Thus, commitment to cell division occurs at the beginning of the cell cycle in rapidly growing cells, but is delayed to later in the cell cycle at slow growth rates. With the appropriate environmental cues these uncommitted cells can undergo an alternative developmental pathway such as conjugation. A similar situation also applies in *S. cerevisiae*¹⁴.

cdc 2 is unique among cell cycle genes in being required independently for two cell cycle events, start and mitosis. Together with *wee 1*, *cdc 2* is involved in the control determining the timing of mitosis^{6–8}. Therefore, *cdc 2* can be considered a cell cycle control gene with regulatory functions at two control points during the cycle—start in G₁ and the timing of mitosis in G₂. Attainment of a critical cell size is required before mitosis can take place^{15–17}. In addition, traverse of start as marked by the completion of *cdc 10* gene function¹³ or the occurrence of S phase^{6–8,18,19} also requires a critical, though different cell size.

Thus, cell size may have an important regulatory role at both cell cycle control points.

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1. Prescott, D. M. *Reproduction of Eukaryotic Cells* (Academic, London, 1976).
2. Pardee, A. B., Dubrow, R., Hamlin, J. L. & Kletzien, R. F. *A. Rev. Biochem.* **47**, 715–750 (1978).
3. Hartwell, L. H. *Bact. Rev.* **38**, 164–198 (1974).
4. Reed, S. I. *Genetics* **95**, 561–577 (1980).
5. Reid, B. J. & Hartwell, L. H. *J. Cell Biol.* **75**, 355–365 (1977).
6. Nurse, P. *Nature* **256**, 547–551 (1975).
7. Thuriaux, P., Nurse, P. & Carter, B. *Molec. gen. Genet.* **161**, 215–220 (1978).
8. Nurse, P. & Thuriaux, P. *Genetics* **96**, 627–637 (1980).
9. Egel, R. & Egel-Mitani, M. *Expl Cell Res.* **88**, 127–134 (1974).
10. Crandall, M., Egel, R. & Macay, V. L. *Adv. microbiol. Physiol.* **15**, 307–398 (1977).
11. Nurse, P., Thuriaux, P. & Nasmyth, K. *Molec. gen. Genet.* **146**, 167–178 (1976).
12. Nasmyth, K. & Nurse, P. *Molec. gen. Genet.* (in the press).
13. Nasmyth, K. *J. Cell Sci.* **36**, 155–168 (1979).
14. Jagadish, M. N. & Carter, B. L. *A. Nature* **269**, 145–147 (1977).
15. Fantes, P. *J. Cell Sci.* **24**, 51–67 (1977).
16. Fantes, P. & Nurse, P. *Expl Cell Res.* **107**, 377–386 (1977).
17. Fantes, P. & Nurse, P. *Expl Cell Res.* **115**, 317–329 (1978).
18. Nurse, P. & Thuriaux, P. *Expl Cell Res.* **107**, 365–375 (1977).
19. Nasmyth, K., Nurse, P. & Fraser, R. J. *J. Cell Sci.* **39**, 215–233 (1979).
20. Thuriaux, P., Sipiczki, M. & Fautes, P. *J. gen. microbiol.* **116**, 525–528 (1980).

Hydrolysis of ATP and reversible binding to F-actin by myosin heavy chains free of all light chains

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Myosin has two 'heads' or subfragments-1 (S-1) each containing a heavy chain, a phosphorylatable or 'regulatory' light chain and another light chain which is generally referred to as being essential^{1–3}. The regulatory light chains can be removed without loss of ATPase activity³, but until now it has not been possible to remove the other class of light chain without complete loss of ATPase activity⁴. However, the dissociating conditions used also denature the heavy chains, and inactivation frequently precedes light-chain dissociation⁵. We now describe the use of mild dissociating conditions combined with affinity chromatography using antibodies to the alkali light chains to produce light-chain-free S-1 heavy chains. The S-1 heavy chain binds reversibly to F-actin and has 30–80% of the ATPase activities of native S-1.

Fast skeletal muscle myosin has two different but related essential light chains, alkali-1 (molecular weight (M_r) = 20,700) and alkali-2 (M_r = 16,500)⁶. Two S-1 isozymes, S-1(A1) and S-1(A2), can be separated on the basis of their alkali light-chain content. These two forms of S-1 seem to have the same heavy-chain compositions, that is, both contain the same two N-terminal sequences⁷. In the absence of actin, S-1(A1) and S-1(A2) have the same ATPase activities. However, at low ionic strengths, the actin-activated ATPase of S-1(A2) has a higher maximal rate of ATP hydrolysis (V_{max})⁸. Using 4.7 M NH_4Cl as a dissociant, purified alkali light chains have been exchanged into S-1 or myosin without inactivation⁹. Similar techniques have been used to prepare hybrids which contain heavy chains from one muscle myosin and 'essential' light chains from a different muscle myosin^{9–11}. Here we have modified the exchange conditions to allow the separation of light chains from heavy chains.

Rabbit antibodies to the alkali light chains of chicken pectoralis muscle myosin were purified and coupled to CNBr-activated Sepharose 4B (3 mg of antibody per ml of Sepharose) as described elsewhere¹². The immunoadsorbent column was eluted in conditions similar to those used in the exchanges, but

Table 1 ATPase activities of S-1 and S-1 heavy-chain preparations

Preparation	EDTA (s ⁻¹)	Ca ²⁺ (s ⁻¹)	Actin-activated			
			<i>I</i> = 0.020 M		<i>I</i> = 0.040 M	
			<i>V</i> _{max} (s ⁻¹)	<i>K</i> _{app} (μM)	<i>V</i> _{max} (s ⁻¹)	<i>K</i> _{app} (μM)
Preparation A						
S-1(A2)	10.1	2.1	25	34		
NH ₄ Cl-treated S-1(A2)*	7.6	1.3	19	26		
S-1 heavy chain (10% S-1(A2))†	7.9	0.9	13.5	28		
Preparation B						
S-1(A2)	9.6	0.96	28	45	24	97
NH ₄ Cl-treated S-1(A2)*	6.5	0.58	20	53	16	110
S-1 heavy chain (15% S-1(A2))†	4.3	0.36	9.4	32	10	89
S-1(A1)	9.5	0.87	16	7.6	23	65

ATPase assays were done using a pH stat to measure the rate of H⁺ release at pH 8.0 and 25° C. Assay conditions for the EDTA-ATPase were: 2 mM EDTA, 5 mM ATP and 0.6 M KCl; for the Ca²⁺-ATPase: 10 mM Ca²⁺, 5 mM ATP and 0.6 M KCl; and for the actin-activated ATPase: 2 mM MgCl₂, 1 mM ATP and 12 or 32 mM KCl. The actin-activated ATPases were analysed using the following equation¹⁸: $v = V_{\max} [\text{actin}] / (K_{\text{app}} + [\text{actin}])$. (*K*_{app} is the concentration of actin required to achieve 1/2 *V*_{max}.)

* S-1(A2) was diluted to 0.25 mg ml⁻¹ with 4.6 M NH₄Cl, 2 mM ATP, 2 mM DTT, 2 mM EDTA and 0.10 M imidazole, pH 7.0 at 4° C. After 1 h, MgCl₂ was added to 5 mM and the S-1(A2) dialysed as described for the light-chain-depleted samples.

† S-1 heavy-chain samples were prepared as described in Fig. 1 legend. The amount of alkali-2 light chain in the samples was determined by densitometry of Coomassie brilliant blue-stained SDS-polyacrylamide gels. The two different S-1 heavy-chain preparations shown indicate the variability in the EDTA-ATPase activities.

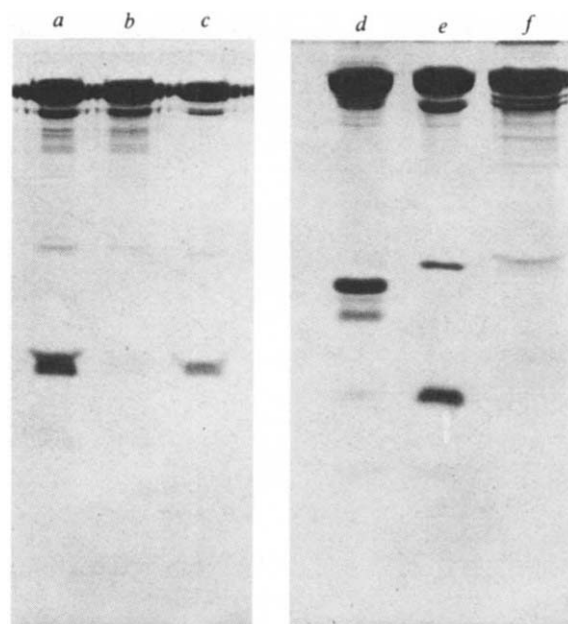
2 mM ATP was added to stabilize the heavy chains during the 1-h isolation procedure and MgCl₂ was added to 5 mM before the removal of the NH₄Cl.

When S-1(A2) was applied to the column, the protein eluting in NH₄Cl contained 85–90% S-1 heavy chain and 10–15% S-1(A2) (Fig. 1). (The phosphorylatable light chains were removed during the digestion of myosin to S-1.) After removing the NH₄Cl, the light-chain-depleted samples had 45–80% of the EDTA-ATPase and ~40% of the Ca²⁺-ATPase of native S-1(A2) (see Table 1). This decrease in ATPase activity may be due in part to the NH₄Cl treatment. In the exchange experiments, where the S-1 was 2.3 mg ml⁻¹, there was only a 10–15% loss in activity⁹. However, when S-1 was left in NH₄Cl for 1 h at

0.25 mg ml⁻¹, equivalent to the S-1 heavy-chain concentration which elutes from the immunoabsorbent column, there was a 25–40% decrease in ATPase activities (Table 1).

When an equal mixture of S-1(A2) and S-1(A1) was applied to the immunoabsorbent column in the same conditions as for Fig. 1, the eluate contained no alkali-2 light chains and half the level of alkali-1 present in the applied S-1. This sample had 73% of the EDTA-ATPase and 45% of the Ca²⁺-ATPase of native S-1. There are two obvious explanations for the preferential removal of the alkali-2 light chain by the immunoabsorbent column: either these particular antibodies interact more strongly with the alkali-2 than with the alkali-1 light chains, or the latter bind more tightly to the S-1 heavy chains than do alkali-2

Fig. 1 SDS-polyacrylamide gel electrophoresis of S-1 heavy-chain preparations. S-1 was prepared by an α-chymotryptic digestion of chicken pectoralis myosin and fractionated into S-1(A1) and S-1(A2)⁸. S-1(A2) (5.0 mg) was incubated for 5 min at 4° C in 1 ml of 4.7 M NH₄Cl, 5 mM ATP, 2 mM EDTA, 2 mM dithiothreitol (DTT) and 0.10 M imidazole, pH 7.0, and then loaded on to a 1.6 × 5 cm immunoabsorbent column (containing antibodies specific for alkali light chains) equilibrated in 4.6 M NH₄Cl, 2 mM ATP, 2 mM DTT, 2 mM EDTA and 0.10 M imidazole, pH 7.0. The column was eluted with this equilibration buffer at 20 ml h⁻¹ and 2.5-ml fractions were collected. The two peak fractions, 0.2 to 0.3 mg ml⁻¹, were pooled and MgCl₂ added to a concentration of 5 mM; 4 ml were dialysed against 0.2 M KCl, 2 × 10⁻⁴ M DTT and 5 mM imidazole, pH 7.0 at 4° C, for use in the ATPase assays, and 1 ml was dialysed against 20 mM NaCl, 2 × 10⁻⁴ M DTT and 5 mM imidazole, pH 7.0 at 4° C, for SDS-polyacrylamide gel electrophoresis. The immunoabsorbent column was regenerated by washing rapidly with 4 M guanidine-HCl. The capacity of the column decreased with use; after six runs only two-thirds as much S-1 could be applied. An A₂₈₀^{1%} of 7.5 and a molecular weight of 115,000 was used for S-1(A2) and for S-1 heavy chain these values were estimated to be 8.6 and 95,000. SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli¹⁷. a, 50 μg of S-1(A2); b, 50 μg S-1 heavy chain, eluted from the immunoabsorbent column in NH₄Cl; c, 25 μg of S-1(A2); d, 50 μg S-1(A1); e, 50 μg S-1(A2) and f, 50 μg S-1 heavy chain as in b, except that it was further purified with the immunoabsorbent column equilibrated in 0.2 M KCl. S-1 heavy chain containing 20% contaminating S-1(A2), 11 ml at 0.25 mg ml⁻¹, was concentrated by ultrafiltration to 2 ml at 1.4 mg ml⁻¹ and applied to the immunoabsorbent column equilibrated in 0.2 M KCl, 2 × 10⁻⁴ M DTT and 5 mM imidazole, pH 7.0 at 4° C. S-1(A2) bound to the column and pure S-1 heavy chain passed through.



light chains. The latter explanation is supported by the observation that the alkali-1 light chain exchanges into myosin more easily than alkali-2 light chain¹⁰. As the alkali-2 light chain is more easily removed from S-1 than alkali-1 light chain, in the rest of the experiments described here, the S-1 and heavy chains were prepared from S-1(A2).

The actin-activated ATPases of both S-1 and S-1 heavy chain have hyperbolic dependences on actin concentration (Fig. 2). In preparation A of Table 1, after correcting for residual S-1(A2), the $V_{\max}$ of S-1 heavy chain was 49% that of native S-1(A2) and 67% that of NH_4Cl -treated S-1(A2). In these assay conditions, the $V_{\max}$ of S-1(A1) is about half that of S-1(A2), but when the ionic strength is increased to 0.04 M, the $V_{\max}$ of S-1(A1) increases to that of S-1(A2)¹³. Preparation B of Table 1 shows the effect of ionic strength on the actin-activated ATPase of the S-1 heavy chain. Although the recovery of actin-activated ATPase was lower than in preparation A, the actin-activated ATPase activities were still much higher than could be accounted for by residual S-1(A2). The $V_{\max}$ of the S-1 heavy chain did not increase significantly when the ionic strength was increased. At both ionic strengths, the K_{app} of the S-1 heavy chain was between those of S-1(A2) and S-1(A1).

Further purification of the heavy chains was achieved by removing residual S-1(A2) on the immunoadsorbent column in the absence of NH_4Cl . No alkali-2 light chain was detectable on SDS-polyacrylamide gels (see Fig. 1). The EDTA-, Ca^{2+} - and actin-activated ATPases of pure S-1 heavy chain were all 30% of those of native S-1(A2). The decreases in activities, most noticeable in the EDTA-ATPase, probably reflect the instability of the heavy chain during the isolation procedures.

To check that the S-1 heavy chains did not contain fragments of the alkali light chains (produced by proteolysis during the isolation procedures) which were not visible on Laemmli-type SDS gels¹⁷, the lysines of an S-1 heavy-chain sample were modified in urea with ^3H -formaldehyde and NaCNBH_3 (ref. 14). The sample was run on an SDS-urea gel capable of resolving small peptides¹⁵. There were no counts in the area of small peptides which could be attributed to digestion products of the alkali light chains nor were any bands detectable by Coomassie blue staining.

The binding of the S-1 heavy chain to F-actin was examined by ultracentrifugation (see Table 2). Centrifugation in the absence of actin showed a slight aggregation of the S-1 heavy chains. (Other experiments also showed that the S-1 heavy chains tend to aggregate more readily than does S-1.) After correcting for aggregation, approximately the same fractions of S-1 heavy chain and S-1(A2) bound to F-actin in the absence of

Table 2 Actin binding of S-1(A2) and S-1 heavy chain preparations

	Fraction bound	
	-ATP	+ATP
S-1(A2)	0.91 (0.02)	0.09 (0.03)
NH_4Cl -treated S-1(A2)	0.82 (0.08)	0.05 (0.03)
S-1 heavy chain (10–15% S-1(A2))	0.82 (0.06)	0.17 (0.09)

S-1 heavy chain or S-1(A2) (1 μM) was mixed with 36 μM F-actin in 0.1 M KCl, 2 mM MgCl_2 , 10^{-4} M DTT and 5 mM imidazole, pH 7.0 at 4° C. Some samples also contained 1.0 mM ATP. After centrifuging the samples at 150,000g for 30 min, the supernatants were carefully removed and aliquots electrophoresed on SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue and the intensity of the heavy-chain bands determined using a scanning gel densitometer. When centrifuged in the absence of actin, 80% of the S-1 heavy chain and 90% of the S-1(A2) and the NH_4Cl -treated S-1(A2) remained in the supernatant. These values were used as references for determining the fraction of S-1 bound to F-actin. Values are the mean ($\pm$ s.d.) of three different S-1 heavy-chain preparations.

ATP, but a lower fraction of S-1 heavy chain was released by ATP.

These results show that the alkali light chains are not essential for either actin binding or ATP hydrolysis, and that the ATP and actin binding sites are located on the myosin heavy chains. However, the alkali light chains may stabilize the myosin conformation, as the heavy-chain preparations seem to be unstable. It has been reported that the isolated heavy chain of the non-filamentous *Acanthamoeba* myosin 1B is fully active¹⁶. As this myosin has many unique properties, it was not possible to extrapolate this observation to other myosins. However, as fast skeletal muscle myosin is a 'typical' myosin, it is likely that the ATP and actin binding sites of other myosins will also reside on the heavy chains.

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- Lowey, S. & Risby, D. *Nature* **234**, 81–84 (1971).
- Sarkar, S., Sreter, F. A. & Gergely, J. *Proc. natn. Acad. Sci. U.S.A.* **68**, 946–950 (1971).
- Weeds, A. G. & Lowey, S. *J. molec. Biol.* **61**, 701–725 (1971).
- Dreizen, P. & Gershman, L. C. *Biochemistry* **9**, 1688–1693 (1970).
- Leger, J. J. & Marotte, F. *FEBS Lett.* **52**, 17–21 (1975).
- Frank, G. & Weeds, A. G. *Eur. J. Biochem.* **44**, 317–334 (1974).
- Pope, B. J., Wagner, P. D. & Weeds, A. G. *J. molec. Biol.* **109**, 470–473 (1977).
- Weeds, A. G. & Taylor, R. S. *Nature* **257**, 54–56 (1975).
- Wagner, P. D. & Weeds, A. G. *J. molec. Biol.* **109**, 455–473 (1977).
- Wagner, P. D. *J. biol. Chem.* **256**, 2493–2498 (1981).
- Hollosi, G., Srivastava, S. & Wikman-Coffelt, J. *FEBS Lett.* **120**, 199–204 (1980).
- Holt, J. C. & Lowey, S. *Biochemistry* **16**, 4398–4402 (1977).
- Wagner, P. D., Slater, C. S., Pope, B. & Weeds, A. G. *Eur. J. Biochem.* **99**, 385–394 (1979).
- Jenoft, N. & Dearborn, D. G. *J. biol. Chem.* **254**, 4359–4365 (1979).
- Swank, R. T. & Munkres, K. D. *Analyt. Biochem.* **39**, 462–477 (1971).
- Maruta, H., Gadasi, H., Collins, J. H. & Korn, E. D. *J. biol. Chem.* **253**, 6297–6300 (1978).
- Laemmli, H. K. *Nature* **227**, 680–685 (1970).
- Eisenberg, E. & Moos, C. *Biochemistry* **7**, 1486–1489 (1968).

Erratum

In the letter 'Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur' by R. E. H. Reid, *Nature* **292**, 49–51 (1981), the sentence beginning on the first line of page 51 should read 'Some material in the literature cited⁷ could also be interpreted differently, for example if bone figured by Seitz (Figs 53, 54 of ref. 14) from the theropod *Allosaurus* is thought to show lamellar-zonal structure'.

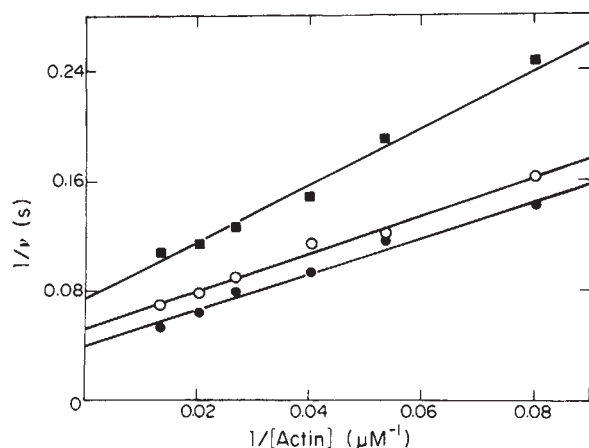


Fig. 2 Actin-activated ATPases. Assays were performed as described in Table 1 legend; v is the observed rate of ATP hydrolysis by S-1 in the presence of actin, minus that of S-1 alone. ●, S-1(A2); ○, NH_4Cl -treated S-1(A2); ■, S-1 heavy chain containing 10% S-1(A2).

BOOK REVIEWS

The case for unilateral disarmament

Hedley Bull

ROBERT Neild, a Professor of Economics at Cambridge and the first Director of the Stockholm International Peace Research Institute, has written a clear-headed and honest little book which will indeed help people to make up their minds about the bomb, even where (like myself) they are not able to accept his conclusions.

In Britain the debate about nuclear weapons policy, dormant throughout the late 1960s and 1970s, has been revived by the recent decisions of the government to purchase the *Trident* missile from the United States and to accept the deployment of United States cruise missiles in Britain. It is to this debate about British policy that Neild seeks to contribute. Does Britain need its own nuclear weapons? And is Britain's security more strengthened than it is endangered by the hosting of United States nuclear bases? To both questions his answers are firmly negative.

Neild holds that while the Soviet Union has developed a "beastly political system" (p.9) and engages in adventurism in the Third World, it does not present a significant military threat in Europe, where its policy is basically defensive. American nuclear weapons deployed in Western Europe, he thinks, would confer positive benefits on the latter only if it could be assumed that the United States is a "reluctant ally" that does not perceive its interests to lie in defending Western Europe. In fact, according to Neild, the United States can be relied upon to defend its investments and world role in Europe, whether or not it has nuclear weapons deployed there that will draw it into any conflict.

The presence of nuclear weapons on British soil, whether British or American, adds to the risk that Britain will be subject to nuclear attack, a danger which official policy is reluctant to acknowledge. As Neild says:

In any nuclear conflict between the United States and the Soviet Union, Britain runs the risk of being the victim of a pre-emptive strike or accident. This is what happens to aircraft carriers [p.18].

Britain's nuclear deterrent force, Neild thinks, expresses

the folly of a declining power which seeks influence on the one hand by ingratiating itself with the United States and becoming dependent on her and, on the other hand, by buying and maintaining nuclear symbols of independence

How to Make Up Your Mind About the Bomb. By Robert Neild. Pp.144. ISBN 0-233-9382-6. (André Deutsch: 1981.) Pbk £2.95.

from the United States, symbols for which it has no use since it does not seek to be independent [pp.99-100].

United States assistance to the British nuclear weapons programme, as Neild notes, has had two conditions: that British nuclear forces be assigned to Nato, and that Britain continues to provide the United States with nuclear bases and other military facilities. Britain's role as an American military base — a role that has been played continuously since the stationing of American bombers in connection with the Berlin Blockade in 1948 — is thus in part made necessary by the British nuclear weapons programme.

The network of American military bases in Britain is now vast (Neild cites a study that lists 103 "bases and facilities"). There has been extraordinarily great governmental secrecy, and extraordinarily little public debate, about their nature and implications. And it is clear that such control as Britain exercises over the use of them, including that of the nuclear-armed bombers now based in Britain, is purely political and informal in nature; nor will the nuclear-armed cruise missiles to be deployed in the next few years be subject to any "double key" system that will give Britain a share in their technical control, such as it has over Nato tactical nuclear weapons deployed on the Continent. This is the measure of the change that has taken place in Anglo-American relations since the Quebec Agreement of 1943, when each country undertook not to use nuclear weapons against any third party without the consent of the other.

Neild is right to insist that it is important to stand back from detailed strategic analysis and ask where the nuclear arms race is going. He is right also to see new dangers in the collapse of super-power arms control negotiations, the drift away from "simple deterrence" towards nuclear war-fighting in strategic doctrine and the tendency to substitute considerations of the balance of nuclear arms between the super powers for considerations of mutual nuclear deterrence. He has performed a service in calling for public debate about US military bases in Britain; many who do not support Neild's view that these should

be removed *in toto* will nevertheless feel, as I do, that given the present direction of United States foreign policy, Britain should be seeking to disengage from any close identification with it, and especially to assert positive control over the use of American nuclear weapons based in Britain.

But, like many rational men of science, Robert Neild does not recognize the part that is necessarily played in international relations by power, including military power. If it is true, as I expect it is, that Soviet leaders harbour no aggressive intentions towards Western Europe, it is also true that the balance of military power created by Nato forms part of the context in which Soviet intentions have taken shape. If the balance were replaced by a Soviet military preponderance in Europe, Soviet leaders would be likely to develop different intentions, if not to invade Western Europe, then at least to dominate it. "Finlandization", which Neild dismisses as a bogey, seems to me to be a very possible consequence of a lowering of the guard by the Western powers, even if the analogy with Finland is an inexact one. Similarly, if the Soviet military position in Eastern Europe were to weaken, pressures would be likely to develop in the Western world to adopt a "forward policy" of promoting change in those countries.

Neild, it is true, does not call for Britain's exit from Nato or for the withdrawal of British forces from Germany; rather, he wants Britain to adopt a "French" policy of national self-reliance in defence, but continued participation in the alliance, except that Britain, unlike France, would be without its own nuclear weapons. Neild, moreover, clearly thinks that Western Europe, even after it had expelled American nuclear weapons, could still look to the United States for military support. He does not consider whether it is morally justifiable for Western European nations to ask the American people to accept on their behalf the risks of having nuclear weapons on American soil, while refusing to accept these risks themselves.

While Britain and other West European countries at present need to distance themselves from an apparently reckless American foreign policy, they are still in danger of being dominated by the Soviet Union if a balance of power in Europe is not maintained. The long-run objective, for Western Europe, should be to create a military counterpoise to the Soviet Union

from its own resources, independently of the United States. But this requires a nuclear deterrent force or forces controlled by West Europeans themselves, for which the British and French nuclear forces now provide a basis, as is today increasingly recognized elsewhere in Western Europe (the West German government, for example, has officially welcomed the British decision to deploy *Trident*). And in

the short run there can be no balance in Europe without the commitment of American nuclear forces, for which some price will have to be paid and some risks accepted.

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Environmental chemistry: a mixed reaction

Peter S. Liss

The Handbook of Environmental Chemistry. Edited by O. Hutzinger. Three volumes of two parts. Vol.1A *The Natural Environment and the Biogeochemical Cycles* pp.258, ISBN 3-540-09688-4. Vol.2A *Reactions and Processes* pp.307, ISBN 3-540-09689-2. Vol. 3A *Anthropogenic Compounds* pp.274, ISBN 3-540-09690-6. (Springer-Verlag: 1980.) Vol.1A DM98, \$57.70; Vol.2A DM126, \$74.40; Vol.3A DM98, \$57.70.

THESE three volumes are the first part of what we are told in the preface will be a multi-part venture. Part Bs for all the present volumes are promised shortly, with further parts to be published at some later date. Readers are even asked for their suggestions as to what future books might contain! Thus we appear to be at the start of what could become a major exercise in environmental chemistry publishing. At this early stage perhaps it is timely to examine both the achievements of what is now published and see what guidance it gives for future projects of this sort.

Each of the present volumes contains between nine and thirteen chapters, each by different authors. Generally they seem to be aimed at an audience at post-graduate level. The whole is edited by O. Hutzinger who is in the Laboratory of Environmental and Toxicological Chemistry at the University of Amsterdam. He has clearly put in an enormous amount of work to get the venture started and presumably continues to labour mightily on future volumes. Although editorial supervision is obviously important for a worthwhile outcome, the editor is, as always in a project of this kind, almost totally at the mercy of the people who are writing the individual articles. In spite of the fact that the authors are generally well known in their subject areas, the final result here is a series of chapters of very uneven content and quality. Some authors have done a really excellent job in summarizing their allotted topics; conversely, several chapters appear to do considerable injustice to the sub-branch of environmental chemistry which they are supposed to cover. This patchiness seriously limits the value of the whole venture. Presumably, the whole purpose of a handbook is that it can always

be relied on to give the sort of information and understanding one requires. If this reliability is lost by some contributions being sub-standard, then one might as well go back to using a collection of textbooks and journal articles covering more restricted parts of the topic.

Since only Part As are currently available, it is not possible to comment at this stage on the comprehensiveness of the *Handbook* or on problems of overlap or underlap between chapters. The arrangement into three volumes is done in a rather interesting way. Volume 1 contains chapters on the hydrosphere, atmosphere, oceans and soils as well as contributions on aspects of biogeochemical cycling. In this last group the article by P. J. Craig, "Metal Cycles and Biological Methylation", deserves special mention since it is a most informative and well balanced appraisal of the topic, particularly with respect to the routes and importance of methylation reactions.

The second volume is concerned with reactions and processes which transport and modify substances in the environment.

Volume 3 contains nine chapters, each on a particular man-made chemical (or group of chemicals) or natural substance whose behaviour is being affected by anthropogenic activities. The list of chemicals covered so far is plainly far from complete. This treatment by individual substance is perhaps how one might have expected the whole *Handbook* to have been organized. The editor is surely right in adopting the approach of establishing the basic principles of the subject in Vols 1 and 2 before proceeding to a detailed examination of individual chemicals.

As to the future of the *Handbook* the message is clear – vigilance in choice of authors is paramount. Although articles must obviously be written by experts in their subject area, it is more important that they should be prepared to make the effort to do justice to their topic rather than be well known in the field.

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Immunity in fertility

W. D. Billington

Immunological Aspects of Reproduction and Fertility Control. Edited by J. P. Hearn. Pp.253. ISBN 0-85200-265-3/0-8391-4143-2. (MTP/University Park Press: 1980.) £19.95, \$44.50.

WHILE reproductive immunology has undoubtedly come of age as a discipline in its own right, it still bears the scars of its rather uncontrolled upbringing in the hands of enthusiastic but sometimes less than rigorous investigators of a wide range of backgrounds. From the resulting morass of scattered, uneven, occasionally confusing, frequently conflicting data, it is now time to extract the relevant and scientifically sound and to reject the remainder. Reviews in this field must therefore be selective, highly critical and, most emphatically, not merely exhaustive catalogues of information. This volume of ten reviews meets this requirement only in part, with almost the full range of possibilities from the truly excellent, through the generally acceptable, to the frankly deplorable. Readers familiar with the field as a whole will hopefully readily discern these categories but the newcomer will require some guidance.

The coverage of the book is wide, with many, but not all, of the immunological aspects of reproduction and fertility control included. It is really two books in one, with the first section largely examining the maternal-foetal relationships in pregnancy and the disorders and diseases, in particular infertility and pre-eclampsia, which may result from disturbances in the normal system, and the second section exploring the possible avenues leading to the production of contraceptive vaccines based upon reproductively unique antigens. The chapters are very variable in length and approach, some presenting reviews of a general topic and others structured around more specific interests and personal data. The philosophy that an understanding of normal reproductive mechanisms will illuminate both pathological events and the means of intervention, as well as vice versa, is here given reasonable substance, and there are many encouraging signs of advance in this fascinating field.

However, this is not to say that many of the major problems do not remain to be solved. Despite considerable endeavour it is still not known by what means the foetus, or more precisely the placenta, avoids rejection as an intra-uterine allograft, whether simply by failing to display appropriate target antigens or by complex mechanisms involving one or more of a diverse range of maternal cellular and humoral immunoregulatory factors. There is conclusive evidence for a primary immunological causation in very few clinical disorders of reproduction. An

acceptable form of contraceptive vaccine is also still awaited, since the most advanced contender, the placental protein hormone chorionic gonadotrophin (hCG), has demonstrated the fundamental difficulty of this approach: that of achieving effective levels of non-cross-reacting, high-affinity antibody in individuals of varied immune responsiveness with preparations of such low immunogenicity as to require the use of potentiating carrier agents and undesirable adjuvants, to say nothing of the possible side-effects. It is questionable whether three entire chapters should have been devoted to this specific molecule, although they certainly highlight the hopes and fears of those working in this important area, illustrated also by two further chapters giving consideration to the potential use of a hypothalamic releasing hormone and antigens of the zona pellucida. It is a pity that the spermatozoal

antigens of relevance in this context should have been relegated to the depths of the lengthy review by Jones on male and female infertility.

This book deserves to be read because it is a fair reflection of the current state of the art, warts and all. But if your time or interest is limited then my advice is to read only the conclusions to each chapter and then go on to digest in full those by Cooper, on immunological relationships between mother and conceptus, and by Redman, on immunological aspects of pre-eclampsia. Both show how it is possible to produce a balanced, critical analysis out of a confusion of data and thereby firmly point the way forward to the required goals. Future reviewers please note. □

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A Devonian view of vertebrate evolution

Alec Panchen

Basic Structure and Evolution of Vertebrates. By Erik Jarvik. Vol.1 pp.575, ISBN 0-12-380801-4; Vol.2 pp. 337, ISBN 0-12-380802-2. (Academic: 1981.) Vol. 1 £41, \$94.50; Vol. 2 £24.40, \$56.50.

IN 1958 one of the recipients of the Linnean Society's Darwin-Wallace centenary medal was Professor E.A. Stensiö, who was honoured principally for his contribution to techniques of fossil preparation. His most important contribution was the technique of serial grinding in which specimens (particularly fossil skulls) are ground away to expose a minutely controlled series of sectional views. No specimen then remains, but a series of drawings and photographs are used to make large-scale, wax-plate reconstructions.

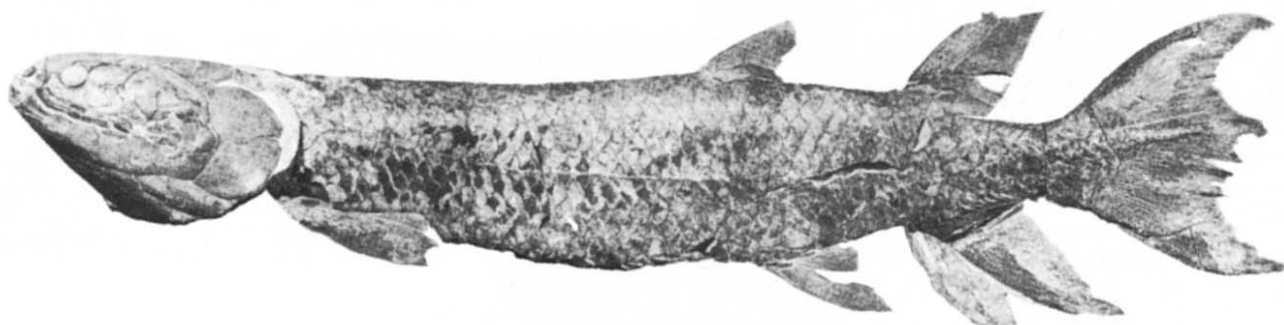
Stensiö may be regarded as the founder of the "Stockholm School" of vertebrate palaeontologists. Professor Jarvik, author of this two-volume work, is his former student and scientific heir, and was, until his retirement a few years ago, Stensiö's

successor in the Chair of Palaeozoology at the Natural History Museum in Stockholm. Thus these volumes record the meticulously detailed comparative anatomy and palaeontology, with numerous, detailed and beautiful line drawings, which characterize the Stockholm School. Unhappily, however, another characteristic feature of the work of Stensiö, Jarvik and their coworkers is very much to the fore: this is due to their isolation, in matters of principle, theory and interpretation, from colleagues elsewhere in the world and particularly from English-speaking palaeontologists. Thus anyone who expects a balanced review of important problems in vertebrate evolution, with views opposed to Jarvik's given due weight, will be disappointed. Not for him is Sir Karl Popper's dictum that the good philosopher or scientist should put his opponent's case in the most convincing manner before proceeding to criticize it.

Following an introduction Jarvik's first two chapters, amounting to 184 pages, are devoted to an uncompromisingly detailed account of the anatomy of two fish, the

primitive extant form *Amia* and the Devonian fossil *Eusthenopteron*. The account of *Amia* is confined to the skeleton and to those features of the "soft anatomy" which can reasonably be restored in a well-preserved fossil skull. The account of *Eusthenopteron* is equally detailed with the "soft anatomy" restored by comparison with *Amia* and other vertebrates. *Eusthenopteron* has for many years been accorded great importance by students of vertebrate phylogeny as the best known representative of a group of fishes standing close to the ancestry of land vertebrates. Jarvik's preoccupation with it has endured throughout his research career, largely as the result of studying one superb skull specimen from a grinding series initiated by Stensiö.

The rest of the first volume is devoted to a review of early fossil vertebrates. Apart from details from living forms and occasional fossils used solely for comparison, none is of later than Devonian age, i.e. less than about 350 million years old. One practical and one theoretical consideration seem to have conditioned this choice: the first is that Jarvik and his present Stockholm colleagues have done little or no work on post-Devonian fossil vertebrates; the second is Jarvik's often-repeated view that all of the most important events in vertebrate evolution were over by the end of the Devonian, so that (with some exaggeration) *Homo sapiens* might be regarded as a aberrant *Eusthenopteron*-like fish! Specialists will find much to admire and also much to disagree with in the details of this review section: Jarvik's heterodox view that both the Dipnoi (lungfish) and the extinct acanthodians are more closely related to sharks than to bony fish will provoke many, as will the views (derived from Stensiö) on the nature of the relationship of early jawless vertebrates to extant cyclostomes. However, perhaps of more general interest is Jarvik's partial fulfilment of a frequently-expressed hope of his colleagues world-wide. Jarvik's department has on loan from the Danish Government an enormous mass of material of the world's earliest known amphibia from the Devonian of East Greenland. The original specimens, deposited in Stockholm over 50 years ago, were



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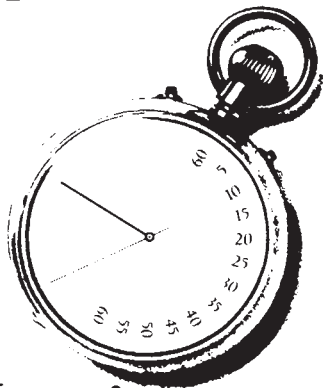
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reviewed by the late G. Säve-Söderbergh in 1932 and since then scraps of information, mostly in the form of annotated drawings, have been released by Jarvik. This review section of his new book yields a few more crumbs in the form of figures and brief descriptions of the limbs and limb girdles, but people will still ask when the definitive monograph on these so-called ichthyostegids is to appear: 50 years is a long time, even in vertebrate palaeontology.

While Jarvik's first volume does deal to some extent with (often heterodox) theories of relationship between groups of fishes, it consists mostly of comparative anatomy and taxonomy. However, the second, shorter, volume is concerned principally with theory. It is divided into four major sections: "Basic structure and composition of vertebrate head" (*sic*), "The origin of the paired extremities", "The origin of tetrapods" and "Recapitulation and comment".

At first glance the opening section appears to be a very old-fashioned treatise on the embryology and anatomy of the skull and associated structures, but there is more to it than that. Jarvik attempts to demonstrate that a much larger part of the cranial anatomy of vertebrates than is normally accepted is not just segmented ("metameric") but actually formed of modified vertebrae — a reversion to the theories of the German *Naturphilosophen* of the early nineteenth century and particularly those of the poet Goethe. In this attempt Jarvik relies heavily on the work of his colleague H.C. Bjerring.

The following section is something of a pleasant surprise in that it opens with a brief but adequate consideration of rival theories of the origin of the paired fins of fishes. Ironically, Jarvik's views on this subject probably represent his most successful challenge to orthodoxy. However, they are followed by a treatment of the origin of the limbs of tetrapods which is strongly coloured by Jarvik's best known and most controversial theory. This is that one group of tetrapods, the Urodela (newts and salamanders) evolved from a different known group of now-fossil fish than did all other tetrapods (the remaining amphibia, reptiles, birds and mammals). Throughout both volumes this theory keeps popping up — sometimes it is assumed in the interpretation of other theories or data, at other times evidence is cited to support it, occasionally with complete circularity of argument. Within the penultimate section of the second volume it is developed in full. It arose originally from Jarvik's study of the snout of *Eusthenopteron* in which he documented a series of anatomical resemblances to the snout of frogs. He then claimed a second series of resemblances connecting members of another group of Devonian fish with the urodeles. Subsequently he has elaborated the comparison, seeking confirmation in other anatomical details. Many workers now

consider the theory to have been refuted by the study of amphibian snouts made by a South African, J.D. Jurgens, but Jarvik does not adequately discuss Jurgens's case.

As a bonus, Jarvik prefaces his discussion of tetrapod origin by a new theory of the mammalian ear in which he derives the three middle-ear ossicles of mammals from the hyoid arch of *Eusthenopteron* and, in rejecting the orthodox homologies, established over 140 years ago, virtually ignores the evidence from the rich source of fossil "mammal-like reptiles".

The final section of the book opens with a chapter on general principles and methods, but does not really address the problem of the irreconcilable methodological differences between the author and his colleagues outside Stockholm. To Jarvik, the correct method of reconstructing phylogeny is the minutely detailed comparison between (inevitably) a small number of ancient fossils and an equally small number of putatively related extant forms. The enormous time gap is ignored in practice and minimized in theory by the assertion that nothing of great evolutionary importance has happened in the past 350 million years. The Anglo-American methodology on the other hand, also often gravely flawed, has been to search for ancestor-descendant sequences, studying, inevitably more superficially, large numbers of fossil species ranged over the whole span of vertebrate history. Perhaps in the future there will be a meeting of minds, also incorporating the less controversial features of cladistic analysis; but Jarvik's book, admirably scholarly in many ways but perverse in many others, will not promote it. □

Alec Panchen, editor of the recent symposium volume The Terrestrial Environment and the Origin of Land Vertebrates (Academic, 1980), is Reader in Vertebrate Zoology at the University of Newcastle upon Tyne.

Nervous molluscs

Steven A. Siegelbaum

Molluscan Nerve Cells: From Biophysics to Behavior. Edited by John Koester and John H. Byrne. Pp.230. ISBN 0-87969-135-2. (Cold Spring Harbor Laboratory: 1981.) \$26, \$31.20 outside the USA.

ONE of the major goals of neurobiologists is to understand how higher processes such as learning and behaviour are related to and controlled by the electrical activity of nerve cells. Until recently, the prevailing view was that what counted most was the pattern of nerve cell connections, the nervous system's wiring diagram, and not primarily the membrane properties of individual nerve cells. However, within the

past few years, a number of researchers have found that, at least for invertebrates, certain aspects of behaviour and "learning" can be explained surprisingly well at the level of the nerve cell membrane's ionic channels.

These recent findings depend, in large part, on our increasingly detailed understanding of the ionic basis of the electrical activity of nerve cells. In 1952, Hodgkin and Huxley demonstrated that the action potential of the squid axon could be understood in terms of two types of ion channels, one permeable to sodium, the other to potassium. While the Hodgkin-Huxley analysis still provides the framework for our present understanding of the electrical activity of nerve cells, it is now clear that no less than five distinct classes of channels exist. These channels play an important role in a variety of physiological processes, ranging from the control of transmitter release from nerve terminals to regulation of the rhythmic patterns of action potential firing produced by nerve cell bodies.

In view of this rather complex description of the nerve cell membrane, this book provides a welcome survey of recent developments in the field. The first in a new series entitled *Cold Spring Harbor Reports in the Neurosciences*, the book is composed of transcripts of talks from a recent symposium which concentrated on studies of molluscan nerve cells. The subjects are arranged in a logical order, starting with a discussion of the biophysical properties of ion channels and progressing to how such channel properties control spike generation patterns and behaviour. The book also includes chapters on the regulation of intracellular ions and the membrane effects of neurotransmitters and hormones.

The material has been presented clearly and at a level which should be accessible to readers with no direct experience in the field, while at the same time informative to those more familiar with the subject. The book contains two introductory chapters (by Eric Kandel and Charles F. Stevens) and should be especially useful for graduate students. One, perhaps inevitable, shortcoming of a book with such a wide scope is that some topics are treated rather briefly and uncritically. This is particularly noticeable in areas where some controversy remains, such as the control of channel opening and closing by internal calcium ions and the mechanism of neurotransmitter modulation of the action potential. Still, the book is an excellent summary and introduction to recent findings in the study of molluscan nerve cells and provides an interesting example of how membrane biophysical techniques may be successfully applied to a wide range of biological questions.

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A question of the evolution of culture

Robert Attenborough

Man, The Promising Primate: The Conditions of Human Evolution. By Peter J. Wilson. Pp.185. ISBN 0-300-02514-9. (Yale University Press: 1980.) \$12.95, £9.10.

A NINETEENTH-century anthropologist would perhaps have found it unsurprising to read that an author's aim was to "consider human evolution from the point of view of social anthropology". That this aim, which Professor Peter Wilson declares at the outset of his book, does sound a little odd today is a measure of the gulf which has developed over the years between social and biological anthropology, making it conventional now for social anthropologists to leave problems of the evolutionary past to their biological colleagues.

Wilson, no doubt rightly in principle, will not allow such conventions to deter him from pursuing his interest in early human societies, and in doing so he meets head-on the main practical obstacle to his ambition: as he puts it, "social factors leave behind no evidence that can serve as a concrete subject of study". So readers are warned: what follows is, as the author frankly says, a purely speculative argument, and anyone who expects much in the way of facts will be disappointed.

Taken on its own terms, then, what is the nature of this argument? Wilson recognizes the value of the Darwinian perspective; he is willing to imagine that human societies must once have been more like those of apes and monkeys than they now are; and he acknowledges that the human capacity for culture must ultimately be an evolved, genetically based characteristic. But how, he asks, can we explain the emergence of this characteristic in a particular primate genus at a particular period and place? It is a good question, and, although he is by no means the first person to ask it, there is still room for fresh ideas.

The basis of Wilson's ideas is the biological proposition that primates are the most primitive and generalized of mammals, and that human beings are in turn the most primitive and generalized of primates. Lacking the evolved specializations which equip so many other animals for their particular ways of life, human beings must be flexible, they must be ready to make the best of whatever conditions they encounter, and they must do so by choosing from a range of options, rather than by giving standard responses: they must, in fact, be capable of dealing with varied day-to-day conditions of material and social life in ways that are too complicated to be genetically programmed in any specific way. From this need, which is both a constraint and a source of freedom, arose the exceptional characteristically human

specialization, the large brain: and with that came the capacities for thought, consciousness and ultimately culture. Human beings are thus "promising primates", creatures of endless potential.

In all this, the focus of Wilson's attention is, naturally, on the social implications. From the generalization of sexual behaviour, he suggests, arises a longer-term mutual interest between males and females, which must be controlled by rules and bonds if it is not to lead to social chaos. The conjunction of the male-female bond with the female-infant bond forms the basis of a third bond between males and infants, and thus the cultural beginnings of kinship, the incest taboo and marriage. But it also creates a social problem, especially for the female, who plainly cannot maintain two close bonds at once. She must divide her attention and her time, and be able to convey the assurance that a neglected relationship will be returned to. Thus human beings must be "promising primates" in a second sense, committing themselves to the future of social relationships.

What, then, should one make of an argument like this? At times, Wilson's advocacy is admirable: serious but also stimulating, ingeniously argued, willing to recognize both primate capabilities and human peculiarities, and (not least of its virtues) well written and not too long. Certainly it deserves the attention of anyone interested in the field. But ultimately it is not altogether satisfying, mainly because its virtues are not maintained consistently. Sometimes Wilson labours too hard over a step in the argument, and the style suffers. And frequently he raises points of detail which tempt the reader to stop and quibble. Moreover, not all points of disagreement are mere details: let me give two illustrations.

The first concerns the use of the concept of generalization. There is, of course, more than a little truth in the notion of human beings as a biologically generalized species (though not in the idea that generalized is synonymous with primitive). But Wilson presses the concept of generalization much further than a biologist would be happy to, relying on it almost as though it were a physical law, a force which, once invoked, carries all aspects of anatomy and behaviour irresistibly along in a single current of evolutionary change. This takes Wilson away from his own correct insis-

Principles of Optics

Pergamon Press have recently published a new edition of *Principles of Optics* by Max Born and Emil Wolf. The latest edition, the sixth, incorporates revisions to the text and illustrations as well as references to the recent literature. Prices are: hbk £22, flexi £12.

tence on understanding the underlying conditions of human evolution. It also creates difficulties for his argument: most notably, it leads him to treat sexual generalization (i.e. continuous female receptivity) as a given feature of the human species, to which other features must adapt, rather than as a phenomenon to be explained in itself.

The second problematic issue is related to the first. Wilson is fairer-minded than many in the capacities that he is willing to attribute to the non-human primates: but, perhaps because his argument emphasizes their relative specialization, he does not go far enough. To take again the example of sex and bonding, he not only doubts the existence of primate sex drives, he also implies that primates do not have multiple social relationships to sustain, as early

human beings, he postulates, did. There is surely no shortage of evidence to refute this suggestion: and if one concedes this point, then Wilson's explanation of why human capacities emerged when and where they did must lose much of its force.

With flaws such as this, Wilson's argument is far from being fully persuasive, even at the frankly speculative level which he intends. But he has taken more trouble than many to present a balanced view of early human societies and, despite its less than total success, his book deserves to be read by anyone with an interest in its theme.

Robert Attenborough is a Lecturer in Biological Anthropology in the Department of Prehistory and Anthropology, Australian National University, Canberra.

Electrochemistry, principles to practice

A.J. Nozik and R.J. Gale

Electrochemical Methods: Fundamentals and Applications. By A.J. Bard and L.R. Faulkner. Pp.718. ISBN hbk 0-471-05542-5; ISBN pbk 0-471-08753-X. (Wiley: 1981.) Hbk £21, \$39.85; pbk £11.75, \$21.70.

THE field of electrochemistry is currently undergoing a major renaissance, now having a central role in such exciting research areas as photoelectrochemistry, chemically modified electrodes, novel energy conversion systems and medical electronics. These fast-moving disciplines all involve a high degree of interaction between solid state physics, physical chemistry, photochemistry, surface science, engineering and electrochemistry. The appearance of *Electrochemical Methods*, which provides a clear and thorough account of all aspects of its subject, is thus timely.

Covering both basic principles of electrochemistry and all the modern and relevant electrochemical and electroanalytical techniques; it is truly an exceptional resource for both practising electrochemists and those eager to learn about this frequently intimidating subject. In addition to the expected material on electrochemical principles and techniques, the book also has a nice, concise introductory chapter on spectroelectrochemistry and photoelectrochemistry; this includes material on optical, electron and ion spectrometry and related phenomena.

The audience for the book will be large since it will also serve as a textbook for senior undergraduate and beginning graduate students. It is the first comprehensive text to cover electroanalytical theory and techniques since Lingane's (*Electroanalytical Chemistry*; Interscience, 1958) and Delahay's (*New Instrumental Methods in Electrochemistry*; Interscience, 1954)

books of the 1950s. Since then, the subject has grown enormously and the range of modern techniques has necessitated a re-evaluation of the emphasis in applications. This has been successfully achieved in this text, and it should prove to be of considerable value in advanced courses in electroanalytical chemistry.

The omission of laboratory procedures is not especially troublesome since this area is complemented by Adams' *Electrochemistry at Solid Electrodes* (Dekker, 1969) and Sawyer and Roberts' *Experimental Electrochemistry for Chemistry* (Wiley, 1974). The absence of the more "classical" electrochemical topics could be a problem as most electroanalytical courses require at least a review of this material. It might also have been more appropriate to introduce concepts of the double-layer at an earlier stage than Chapter 12; Chapter 9 should be singled out as an excellent introduction to impedance concepts. The emphasis and presentation of the subject material are superlative throughout and the text could not be improved without considerable expansion in size and cost. An additional bonus is the problems, which provide useful exercises in the more important concepts and topics.

Very few errors have been noted; however, Problem 9.4 (p.368) does not tabulate the frequency as indicated, and the axes in Figure 14.8.2 (p.655) are labelled in an ambiguous (unconventional) manner.

This text has filled a definite need in present-day electroanalytical literature. We recommend it highly to students, teachers and research scientists.

A.J. Nozik is Chief of the Photoconversion Research Branch at the Solar Energy Research Institute, Golden, Colorado, and R.J. Gale is an Assistant Professor in the Department of Chemistry at Louisiana State University, Baton Rouge.

Ionic solutions

A.K. Covington

Ionic Liquids. Edited by D. Inman and D.G. Lovering. Pp.450. ISBN 0-306-40412-5. (Plenum: 1981.) \$49.50, £31.19.

MOST conference volumes are conceived before the meeting itself takes place, and these days contributors are expected to arrive with camera-ready copy or place their expenses in jeopardy. The decision to publish the papers from this conference organized jointly by the Electrochemistry and Molten Salts Discussion Groups of the Chemical Society (now the Royal Society of Chemistry) apparently arose during the conference at St John's College, Oxford, in July 1978. The intention of the meeting organizers was to provide a greater exchange of ideas between aqueous solution chemists and molten salt chemists, thereby focusing attention on the somewhat neglected area of the properties of concentrated electrolyte solutions.

As with most conference volumes, the quality, approach and style of the 20 presentations are variable. Particularly valuable, however, are Enderby's account of the important neutron scattering first order difference method of determining radial distribution functions, and hence cation and anion hydration in concentrated solutions such as $\text{NiCl}_2/\text{D}_2\text{O}$, $\text{CaCl}_2/\text{D}_2\text{O}$, Duffy and Ingram's account of acid-base properties of concentrated aqueous solutions, and Adams and Hills's perceptive survey of computer simulation methods. The latter authors are somewhat scathing in their criticism of the preoccupation of solution chemists with continuum theories. In this connection, one of the contributors (Richter) quotes a 1957 paper of Fuoss and Onsager who thought that the problem of concentrated salt solutions should be approached from the fused salt side. Little progress has been made in those 24 years although recent approaches by Blander (Argonne), who was not a contributor, look promising.

Several papers highlight problems of technological interest — Dead Sea brines (Marcus), aluminium halide containing melts (Osteryoung) and sulphur and sulphide species in melts (Plichon) — and restore the balance towards practical aspects. Four papers deal with various aspects of water in melts: industrial and electrochemical (Lovering), concentration dependence of transport properties (Claes), solution chemistry (Coombes), and effect on purification (White).

The book is excellently produced with good, clear diagrams. It is to be recommended with the hope that it will, by placing the problems in perspective, encourage that progress which was the aim of the organizers.

A.K. Covington is Reader in Physical Chemistry at the University of Newcastle upon Tyne.

BOOKS RECEIVED

Applied Biological Sciences

GEBELEIN, C. G. and KOBLITZ, F. F. Biomedical and Dental Applications of Polymers. Polymer Science and Technology, Vol.14. Based on an American Chemical Society Symposium held March 1980 at the 179th National Meeting in Houston, Texas. Pp.492. ISBN 0-306-40632-2. (Plenum: 1981.) \$59.50.

GORNALL, A. G. (ed.). Applied Biochemistry of Clinical Disorders. Pp.444. ISBN 0-06-141010-1. (Lippincott-Harper, Philadelphia: 1981.) \$30.

HILLENKAMP, F., PRATESI, R. and SACCHI, C. A. (eds). Lasers in Biology and Medicine. NATO Advanced Study Institutes Series. Series A, Life Sciences. Proceedings of the NATO Symposium on Lasers in Biology and Medicine, Camaiore, Lucca, Italy, August 1979. Pp.463. ISBN 0-306-40470-2. (Plenum: 1980.) \$49.50.

JENKYN, J. F. and PLUMB, R. T. (eds). Strategies for the Control of Cereal Disease. Pp.219. ISBN 0-632-00716-8. (Blackwell Scientific: 1981.) £13.

MIZRAHI, A. *et al.* (eds). New Developments with Human and Veterinary Vaccines. 25th OHLO Biological conference, Zichron, Ya'acov, Israel. Pp.444. ISBN 0-8451-0047-5. (Alan R. Liss: 1980.) \$36, Dfl.108.

PETRIE, J. C. (ed.). Cardiovascular and Respiratory Disease Therapy. Clinically Important Adverse Drug Interactions, Vol.1. Pp.243. ISBN 0-444-80233-9. (Elsevier/North-Holland Biomedical: 1980.) \$48.25, Dfl.99.

RAINERI, A., KELLERMAN, J. J. and RULLI, V. (eds). Selected Topics in Exercise Cardiology and Rehabilitation. Ettore Majorana International Science Series. Life Sciences, Vol.4. Proceedings of the 1st Course held in Erice, Sicily, October 1979. Pp.279. ISBN 0-306-40566-0. (Plenum: 1980.) \$35.

RUBENSTEIN, I. *et al.* (eds). Genetic Improvement of Crops. Emergent Techniques. Pp.242. ISBN 0-8166-0966-7. (University of Minnesota Press: 1980.) \$22.50.

SAKAGUCHI, K. and OKANISHI, M. (eds). Molecular Breeding and Genetics of Applied Microorganisms. Pp.160. ISBN 0-12-615050-8. (Academic: 1981.) \$27.

SERROU, B. and ROSENFELD, C. (eds). Immune Complexes and Plasma Exchanges in Cancer Patients. Human Cancer Immunology, Vol.1. Pp.344. ISBN 0-444-80237-1. (Elsevier/North-Holland Biomedical Press: 1981.) \$87.75, Dfl. 180.

SKINNER, F. A., PASSMORE, S. M. and DAVENPORT, R. R. (eds). Biology and Activities of Yeasts. The Society for Applied Bacteriology Symposium Series, No. 9. Pp.310. ISBN 0-12-648080-X. (Academic: 1981.) £15.80, \$36.50.

VAINIO, H., SORSA, M. and HEMMINKI, K. (eds). Occupational Cancer and Carcinogenesis. Pp.422. ISBN 9-89116-193-7. (Hemisphere Publishing: 1981.) \$49.50.

WAGNER, J. C. (ed.). Biological Effect of Mineral Fibres, Vol.2. Proceedings of a Symposium held at the International Agency for Research on Cancer, Lyon, France, September 1979. Pp.1007. ISBN 2-85598-199-9. (International Agency for Research on Cancer, Lyon: 1980.) SwFr. 60, \$35.

WALKER, E. A. *et al.* (eds). N-Nitroso Compounds: Analysis, Formation and Occurrence. Proceedings of the VIth International Symposium held in Budapest, October 1979. Pp.841. ISBN 92-8-321131-6. (International Agency for Research on Cancer, Lyon: 1980.) SwFr. 70, \$40.

ZWEIG, G. and SHERMA, J. (eds). Updated General Techniques and Additional Pesticides. Analytical Methods for Pesticides and Plant Growth Regulators, Vol.XI. Pp.408. ISBN 0-12-784311-6. (Academic: 1980.) \$46.

Psychology

FRANSELLA, F. (ed.). Personality. Theory, Measurement and Research. Pp.256. Hbk ISBN 0-416-72770-0; pbk ISBN 0-416-72780. (Methuen: 1981.) Hbk £10.50; pbk £4.95.

FRIEDMAN, S. L. and SIGMAN, M. (eds). Preterm Birth and Psychological Development. Pp.438. ISBN 0-12-267880-X. (Academic: 1981.) \$34.

GRISIO, T. Juveniles' Waiver of Rights. Legal and Psychological Competence. Perspectives in Law & Psychology, Vol.3. Pp.295. ISBN 0-306-40526-1. (Plenum: 1981.) \$32.50.

HERSEN, M., EISLER, R. M. and MILLER, P. M. (eds). Progress in Behavior Modification, Vol.10. Pp.243. ISBN 0-12-535610-2. (Academic: 1980.) \$27.

KENDAL, P. C. and HOLLON, S. D. (eds). Assessment Strategies for Cognitive-Behavioral Interventions. Personality and Psychopathology. Pp.425. ISBN 0-12-404460-3. (Academic: 1980.) \$29.50.

LEWIS, H. B. Freud and Modern Psychology. Vol.1, The Emotional Bases of Mental Illness. Emotions, Personality and Psychotherapy. Pp.247. ISBN 0-306-40525-3. (Plenum: 1981.) \$19.50.

LUCURTO, C. M., TERRACE, H. S. and GIBBON, J. (eds). Autoshaping and Conditioning Theory. Pp.313. ISBN 0-12-454480-0. (Academic: 1980.) \$30.

PALMER, E. L. and DORR, A. (eds). Children and the Faces of Television. Teaching, Violence, Selling. Pp.359. ISBN 0-12-544480-X. (Academic: 1980.) \$24.50.

PIAGET, J. Intelligence and Affectivity: Their Relationship During Child Development. Pp.77. ISBN 0-8243-2901-5. (Annual Reviews, Palo Alto, California: 1981.) \$8 (U.S.A.) \$9 (elsewhere).

REHM, L. P. Behavior Therapy for Depression. Present Status and Future Directions. Pp.389. ISBN 0-12-585880-9. (Academic: 1980.) \$29.50.

SALES, B. D. (ed.). The Trial Process. Perspectives in Law and Psychology, Vol.2. Pp.506. ISBN 0-306-40491-5. (Plenum: 1981.) \$39.50.

TAJFEL, H. Human Groups and Social Categories. Pp.369. Hbk ISBN 0-521-22839-5; pbk ISBN 0-521-28073-7. (Cambridge University Press: 1981.) Hbk £25; pbk £7.95.

VALZELLI, L. Psychobiology of Aggression and Violence. Pp.262. ISBN 0-89004-403-1. (Raven: 1981.) \$26.

WEXLER, D. B. Mental Health Law. Major Issues. Perspectives in Law and Psychology, Vol.4. Pp.270. ISBN 0-306-40538-5. (Plenum: 1981.) \$25.

YENBI-KOMSHIAN, G. H., KAVANAGH, J. F. and FERGUSON, C. A. (eds). Child Phonology. Vol.2, Perception. Perspectives in Neurolinguistics, Neuropsychology and Psycholinguistics. Pp.254. ISBN 0-12-770602-X (Academic: 1980.) \$24.

Sociology

BREZHNEV, L. I. Socialism, Democracy and Human Rights. Pp.247. ISBN 0-08-023605-7. (Pergamon: 1981.) £22.

COOMBS, P. H. (ed.). Meeting the Basic Needs of the Rural Poor. A Report of The International Council for Educational Development. Pp.816. ISBN 0-08-026306-2. (Pergamon: 1980.) £24.75.

History of Science

BOWERS, J. Z. When The Twain Meet. The Rise of Western Medicine in Japan. The Henry E. Sigerist Supplements to the Bulletin of the History of Medicine, New Series No. 5. Pp.173. ISBN 0-8018-2432-X. (The Johns Hopkins University Press: 1981.) \$14.

CASTILLEJO, D. The Expanding Force in Newton's Cosmos As Shown in His Unpublished Papers. Pp.125. Flexi ISBN 84-85005-49-X. (Ediciones de Arte Y Bibliofilia, Madrid: 1981.) Np.

JAHNKE, H. N. and OTTE, M. (eds). Epistemological and Social Problems of the Sciences in the Early Nineteenth Century. Pp.430. ISBN 90-277-1223-9. (Reidel: 1981.) \$31.50, Dfl.60.

KINTAKKA, J., GRUENDER, D. and AGAZZI, E. (eds). Theory Change, Ancient Axiomatics, and Galileo's Methodology. Proceedings of the 1978 Pisa Conference on the History and Philosophy of Science. Vol.1, Synthese Library. Studies in Epistemology, Logic, Methodology, and Philosophy of Science, Vol.145. Pp.348. ISBN 90-277-1126-7. (Reidel: 1980.) \$50.

KINTAKKA, J., GRUENDER, D. and AGAZZI, E. (eds). Probabilistic Thinking, Thermodynamics and the Interaction of the History and Philosophy of Science. Proceedings of the 1978 Pisa Conference on the History and Philosophy of Science. Vol.II, Synthese Library. Studies in Epistemology, Logic, Methodology, and Philosophy of Science, Vol.146. Pp.340. ISBN 90-277-1127-5. (Reidel: 1980.) \$89.50, Dfl.95.

MARK, J. Four Anthropologists: An American Science in Its Early Years. Pp.209. ISBN 0-88202. (Science History Publications, New York: 1980.) \$20.

WALLACE, W. A. Prelude to Galileo. Essays on Medieval and Sixteenth-Century Sources of Galileo's Thought. Pp.369. Hbk 90-277-1215-8; pbk ISBN 90-277-1216-6. (Riedel: 1981.) Hbk Dfl. 95.00, \$49.95; pbk Dfl. 45.00, \$23.50.

General

BACKNELL, H. III. Energy and the National Defense. Pp.235. ISBN 0-8131-0402-5. (The University Press of Kentucky: 1981.) \$19.50.

BAILLAUD, R. Souvenirs D'un Astronome (1908-1977.) Pp.631. (Imprimerie Carrere-12-Rodez: 1980.) Flexi Np.

BALLMER, T. and BRENNENSTUHL, W. Speech Act Classification: A Study in the Lexical Analysis of English Speech Activity Verbs. Springer Series in Language and Communication, Vol.8. Pp.274. ISBN 3-540-10294-9. (Springer-Verlag: 1981.) DM 57, \$33.60.

BARNEY, G. O. (Study Director) The Global 2000 Report to the President of the U.S. Entering the 21st Century. Vol.1, The Summary Report. A Report Prepared by the Council on Environmental Quality and the Department of State. Pp.360. Hbk ISBN 0-08-024617-6; pbk ISBN 0-08-024618-4. (Pergamon: 1981.) £25, \$50 (2 vols.).

BARRACLOUGH, N. Preology. Sociedad Española de Historia Y Filosofía de la Ciencia, Madrid. Pp.260. Flexi ISBN 0-08-026083-7. (Pergamon: 1981.) £5.85, \$14.25.

BATESON, P. P. G. and KLOPFER, P. H. (eds). Perspectives in Ethology. Vol.4, Advantages of Diversity. Pp.249. ISBN 0-306-40511-3. (Plenum: 1981.) Np.

BAYNES-COPE, A. D. Caring for Books and Documents. Pp.32. ISBN 0-7141-2006-5. (British Museum, London: 1981.) £2.50.

BELLAMY, D. and MACKIE, S. The Great Seasons. Pp.153. ISBN 0-340-25720-2. (Hodder & Stoughton: 1981.) £9.95.

BÖGLI, A. Karst Hydrology and Physical Speleology. Pp.284. ISBN 3-540-10098-9. (Springer-Verlag: 1980.) DM 58, \$34.30.

BREZHNEV, L. I. Peace Detente Co-operation. Pp.197. ISBN 0-306-10971-9. Consultants Bureau, New York and London: 1981.) Np.

DURAND-DROUHIN, J.-L. and SZWENGRUB, L.-M. (eds). Rural Community Studies in Europe. Trends, Selected and Annotated Bibliographies. Analyses, Vol.1. Pp.332. ISBN 0-8-021384-7. (Pergamon: 1981.) £30, \$72.

EISENBERG, L. and KLEINMAN, A. (eds). The Relevance of Social Science for Medicine. Culture, Illness and Healing. Vol.1. Pp.398. Hbk ISBN 90-277-1176-3; pbk ISBN 90-277-1185-2. (Reidel: 1980.) Hbk Dfl.65, \$34; pbk Dfl.30, \$14.95.

OQUIST, P. Violence, Conflict, and Politics in Colombia. Pp.263. ISBN 0-12-527750-4. (Academic: 1980.) \$25.

SHAYER, M. and ADEY, P. Towards a Science of Science Teaching. Cognitive Development and Curriculum Demand. Pp.159. Pbk ISBN 435-57825-1. (Heinemann Educational: 1981.) Pbk np.

SIDERI, S. and JOHNS, S. (eds). Mining for Development in the Third World. Multinational Corporations, State Enterprises and the International Economy. Pp.325. ISBN 0-08-026308-9. (Pergamon: 1981.) £17.50, \$35.

ANNOUNCEMENTS

Awards

Dr Paul Rebut (deputy director of JET) has been awarded le Grand Prix de Physique Jean Ricard for 1981 by the Council of the Société Française de Physique (SFP).

The annual Karl Heinrich Gyr and Heinrich Landis Commemorative Prize, which is administered by the Institution of Electrical Engineers, has been awarded to **Mr Martin Weston** of the BBC.

The International Union Against Cancer, with the funds provided by the American Cancer Society, will award Eleanor Roosevelt Fellowships for research on cancer only to persons on the staff of universities, teaching hospitals, research laboratories or similar institutions, and to investigators who are devoting themselves to the experimental or the clinical aspects of cancer research. The Yamagiwa-Yoshida Memorial International Cancer Study Grants are funded by the Japan National Committee for the UICC. These Study Grants are also administered by the International Union Against Cancer. They are designed to enable investigators of any nationality to gain experience in, or make comparative studies of, special techniques in both the biological and clinical aspects of cancer research. Application forms and additional information from: International Union Against Cancer, rue du Conseil-Général, 3, 1205 Geneva, Switzerland.

Twenty Harkness Fellowships tenable for between 12 and 21 months are offered each year. The award includes return fares to the United States, living and family allowances, travel in America (with car rental allowance), tuition and research expenses, a book and equipment allowance and health insurance. Candidacy is open to men and women between 21 and 30 years of any profession or field of study, who are citizens of the UK and have received *both* secondary schooling *and* further education (or equivalent professional experience in lieu of further education) wholly or mainly in the United Kingdom. For application forms: send a s.a.e. carrying 26p postage, and measuring not less than 10 inches by 7 inches, to The Harkness Fellowships (UK), Harkness House, 38 Upper Brook Street, London W1, UK.

Meetings

13-16 October, **Indoor Air Pollution, Health and Energy Conservation**, Massachusetts (Dr J.D. Spengler, Dept of Environmental Health Sciences, Harvard School of Public Health, 665 Huntington Ave, Boston, Massachusetts 02115, USA).

14 October, **Geological Aspects of the Disposal of Radioactive Waste in the Sea**, London (Mineralogical Society, 41 Queen's Gate, London SW7, UK).

18-23 October, **Water Chlorination: Environmental Impact and Health Effects**, California (Dr R.L. Jolley, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830, USA).

19-22 October, **Pattern Recognition**, Munich (Prof. Dr H. Marko, Lehrstuhl für Nachrichtentechnik der Technischen Universität München, 8000 München, FRG).

19-21 October, **Spectroscopic Methods for Biomedical Research**, Columbus (K.L. Waite, Battelle's Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201, USA).

23-24 October, **The Role of Receptors in the Skin**, Nice (Prof. Hans Schaeffer, CIRD, Sophia Antipolis, F-06565 Valbonne, France).

27-30 October, **10th European Symposium on Clinical Pharmacological Evaluation in Drug Control: Drugs for Infants and Children**, Schlangenbad (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

25-28 October, **Laboratory Health and Safety**, Loughborough (Centre for Extension Studies, University of Technology, Loughborough, Leicester, UK).

25 October — 7 November, **Plant Tissue Culture Methods and Applications in Agriculture**, Los Banos (Dr T.A. Thorpe, Dept of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4).

27-29 October, **Polynuclear Aromatic Heterocarbons**, Columbus (D. Cooley, Battelle's Columbus Laboratories, 505 King Ave, Columbus, Ohio 43201, USA).

29-31 October, **Rural Development and Retention of the Rural Population in the Countryside of the Developing Countries**, Ottawa (J. Havet, Institute for International Cooperation, University of Ottawa, 190 Laurier Ave East, Ottawa, Ontario, Canada K1N 6N5).

29-30 October, **Office Health and Safety**, Loughborough (Centre for Extension Studies, University of Technology, Loughborough, Leicester, UK).

31 October — 2 November, **Annual Meeting of the American Society for Cybernetics**, Washington (Dr L.D. Richards, Dept of Administrative Science, Colby College, Waterville, Maine 04901, USA).

3-6 November, **Working Group on Appropriate Technology for Health in Radiology, Radiotherapy and Nuclear Medicine**, Rome (World Health Organization, 9, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

3-6 November, **Health and Safety at Work**, Wembley (Maclaren Exhibitions Ltd, Davis House, 69-77 High St, Croydon, UK).

3-9 November, **Remote Sensing of Arid and Semi-arid Lands**, Cairo (E.S. Kasischke, Environmental Research Institute of Michigan, PO Box 8618, Ann Arbor, Michigan 48107, USA).

4-6 November, **EFOC '81**, Cologne (Information Gatekeepers Inc. c/o Tom Wearden Associates Ltd. 11 Chalmore Gardens, Wallingford, Oxon, UK).

9-11 November, **Mechanical Behavior of Salt**, Pennsylvania (Dr H. Reginald Hardy Jr, Rock Mechanics Laboratory, Room 117 Mineral Sciences Building, The Pennsylvania State University, University Park, Pennsylvania 16802, USA).

7-8 November, **32nd Annual Meeting of the American Association for the Study of Liver Diseases**, Chicago (Charles B. Slack, Inc. 6900 Grove Rd, Thorofare, New Jersey 08086, USA).

9-13 November, **1981 Postgraduate Course in Clinical Pharmacology, Drug Development and Regulation**, New York (Dr W.M. Wardell, Dept of Pharmacology, The University of Rochester Medical Center 601 Elmwood Ave, Rochester, New York 14642, USA).

11 November, **Precambrian-Cambrian Evolutionary Explosion**, Bristol (Dr R. Riding, Dept of Geology, University College, Cardiff, UK).

19-21 October, **Working Group on Legionnaires Disease**, Baden (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

12-13 November, **Advisory Committee on Prophylactic, Diagnostic and Therapeutic Substances**, EURO (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

14-19 November, **Seminar on Appropriate Technology for Drinking Water Demineralization**, Algiers (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

12-13 November, **Common Denominators in Art and Science**, Edinburgh (Prof. M.R. Pollock, Marsh Farm House, Margaret Marsh, Shaftesbury, Dorset, UK).

16-17 November, **HPLC of Proteins and Peptides**, Washington (R. Schulthess, Varian AG, Steinhauserstrasse, 6300 Zug/Switzerland).

17-19 November, **Taking Industry Offshore**, London (IMechE, 1 Birdcage Walk, Westminster, London SW1, UK).

18-19 November, **Business Opportunities in Genetics**, Madison (C.W. Little, University of Wisconsin Extension, Dept of Engineering, 432 North Lake St, Madison, Wisconsin 53706, USA).

16-17 December, **Structure of the Interfacial Region**, Oxford (Dr M. Lal, Unilever Research, Port Sunlight Laboratory, Bebington, Wirral, Merseyside, UK).

17-20 December, **Annual Conference of The Palaeontological Association**, Exeter (Prof. J.W. Murray, Dept of Geology, University, Exeter, UK).